

Supplementary information

Detail analysis of the stability and photoregulation efficiency of the interstrand-wedged duplex

Supplemental Table 1. T_{ms} of duplex **nD₁₂-M/nD₁₂-N** (5'-GCACGMGGTCGG-3' / 5'-CCGACCNCGTGC-3').^{a, b)}

N \ M	A	C	G	T
A	49.5 (37.2)	51.4 (36.6)	55.3 (49.0)	61.0 (61.9)
C	48.1 (35.7)	45.9 (39.1)	62.6 (63.9)	47.6 (38.4)
G	52.7 (44.5)	65.1 (64.0)	54.3 (54.4)	54.5 (50.0)
T	60.3 (62.3)	48.2 (41.1)	52.4 (53.6)	50.6 (53.8)

^{a)} The values in the parenthesis are T_{ms} of corresponding duplex **XD1-M/XD2-N**.

^{b)} Conditions: 3.5 μ M DNA, 100 NaCl, 10 mM NaH₂PO₄ (pH 7.0).

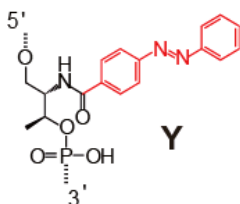
Supplemental Table 2. T_{ms} of duplex 5'-CGTYTAYMGTYTCA-3' / 3'-GCYAAYTNCYAAAYGT-5'.^{a-c)}

N \ M	A	C	G	T
A	41.5 (37.2)	42.1 (36.6)	42.5 (49.0)	55.4 (61.9)
C	40.2 (35.7)	45.9 (39.1)	57.5 (63.9)	41.4 (38.4)
G	41.5 (44.5)	57.3 (64.0)	42.5 (54.4)	45.0 (50.0)
T	55.1 (62.3)	42.1 (41.1)	45.3 (53.6)	47.8 (53.8)

^{a)} The values in the parenthesis are T_{ms} of corresponding duplex **YD1-M/YD2-N**.

^{b)} Conditions: 6 μ M DNA, 100 mM NaCl, 10 mM NaH₂PO₄ (pH 7.0).

^{c)} The structure of non-modified azobenzene (**Azo**) moiety (**Y**) is shown as follows:



Supplemental Table 3. Melting temperatures (T_m s) of DNA duplexes involving either **X (DM-azo)** or **Y (Azo)** residues

Duplex	$T_m / ^\circ\text{C}$ ^{a)}		ΔT_m ^{c)}
	<i>trans</i> ^{b)}	<i>cis</i>	
XD3/XD4	73.3 (10.1)	42.0	31.3
XD3/nD4	68.6 (5.4)	55.5	13.1
nD3/XD4	69.4 (6.2)	53.0	16.4
YD3/YD4	68.9 (6.6)	37.5	32.3
nD3/nD4		63.2	
D3-5T/D4-5T ^{d)}		22.5	

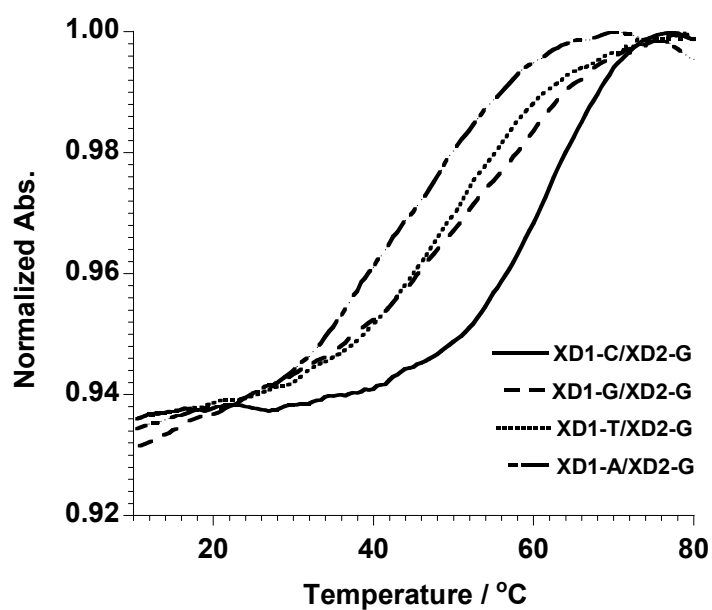
^a Conditions: 2 μM DNA, 100 mM NaCl, pH 7.0 (10 mM phosphate buffer).

^b Values in parenthesis indicate the difference in T_m as compared with native duplex **nD3/nD4**.

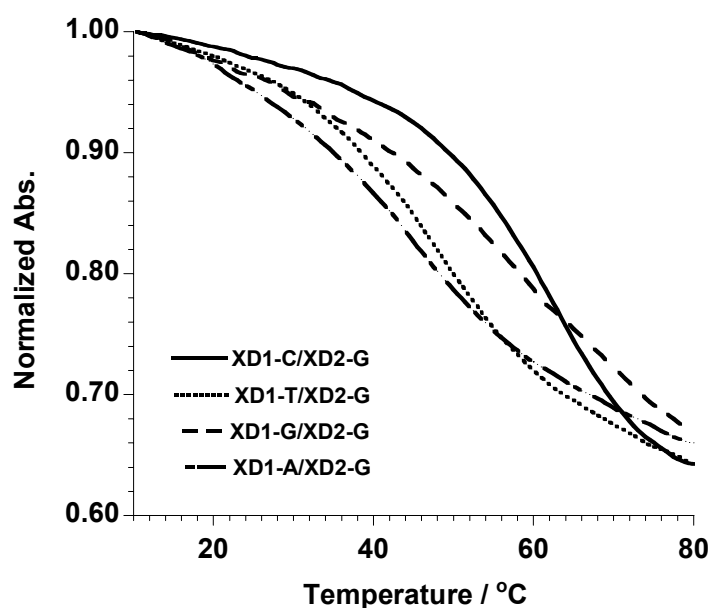
^c Change of T_m induced by the *cis-trans* isomerization.

^d Sequence of duplex **D3-5T/D4-5T** is 5'-GTCTTG TTTT GTCTAC-3'/3'-CAGAAC TTTT CAGATG-5')

cis-**XD3/XD4** showed a higher T_m (~42 $^\circ\text{C}$) than that of duplex **D3-5T/D4-5T** (5'-GTCTTG TTTT GTCTAC-3'/3'-CAGAAC TTTT CAGATG-5'), in which 5 continuous TT mismatches were involved and its T_m was 22.5 $^\circ\text{C}$. On the other hand, when non-modified azobenzene (**Azo**) was used instead of **DM-azo**, *cis*-**YD3/YD4** also showed a higher T_m than that of the duplex involving 5 TT mismatches. *cis*-**XD3/XD4** (**DM-azo**) even showed a higher T_m as compared with *cis*-**YD3/YD4** (**Azo**), in which six **Azos** were used to construct the interstrand-wedged motif instead of **DM-Azos**, probably because the interaction between *cis*-**DM-azos** was stronger than that of *cis*-**Azos**.

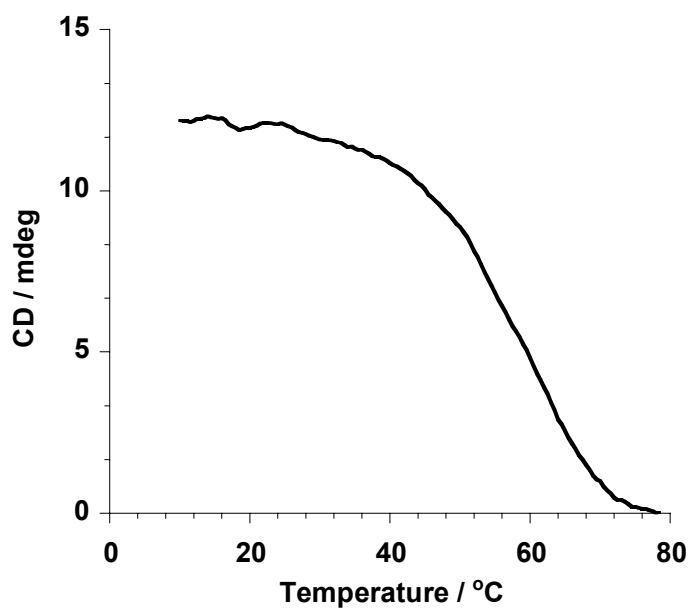


Supplemental Figure 1. Melting curves (260 nm) of duplex **XD1-C/D2-G** (solid line, $T_m = 64.0$ °C), **XD1-G/XD2-G** (dashed line, $T_m = 54.4$ °C), **XD1-T/XD2-G** (dotted line, $T_m = 50.0$ °C), **XD1-A/XD2-G** (dash-dotted line, $T_m = 44.5$ °C). Conditions: 3.5 μ M DNA, 100 mM NaCl, pH 7.0 (10 mM phosphate buffer).

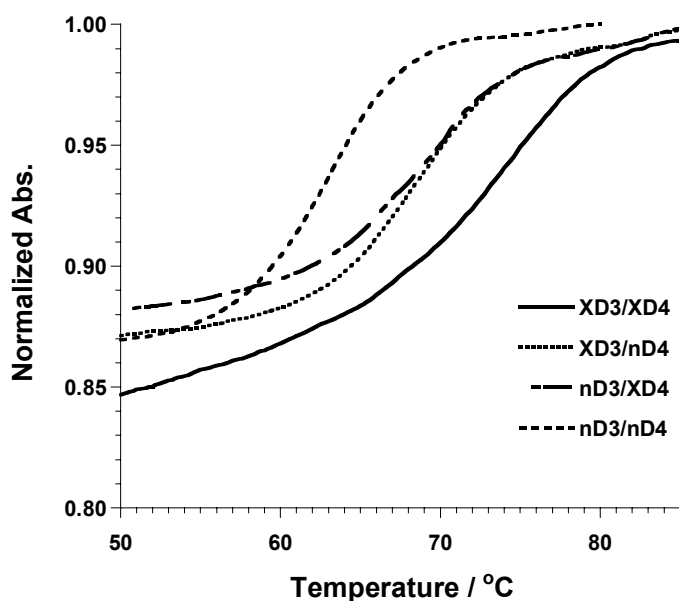


Supplemental Figure 2. Melting curves (350 nm) of duplex **XD1-C/XD2-G** (solid line, $T_m = 62.2^\circ\text{C}$), **XD1-G/XD2-G** (dashed line, $T_m = 55.2^\circ\text{C}$), **XD1-T/XD2-G** (dotted line, $T_m = 49.4^\circ\text{C}$), **XD1-A/XD2-G** (dash-dotted line, $T_m = 44.9^\circ\text{C}$). Conditions: 3.5 μM DNA, 100 mM NaCl, pH 7.0 (10 mM phosphate buffer).

As illustrated in Figure 2, the absorbance at 350 nm decreased with the dissociation of the duplex, which is reversed as compared with that at 260 nm (see Supplemental Figure 1). It is also worth noting that the absorbance at 350 nm increased more than 50% due to the duplex formation, whereas the absorbance at 260 nm decreased only 6-7%.

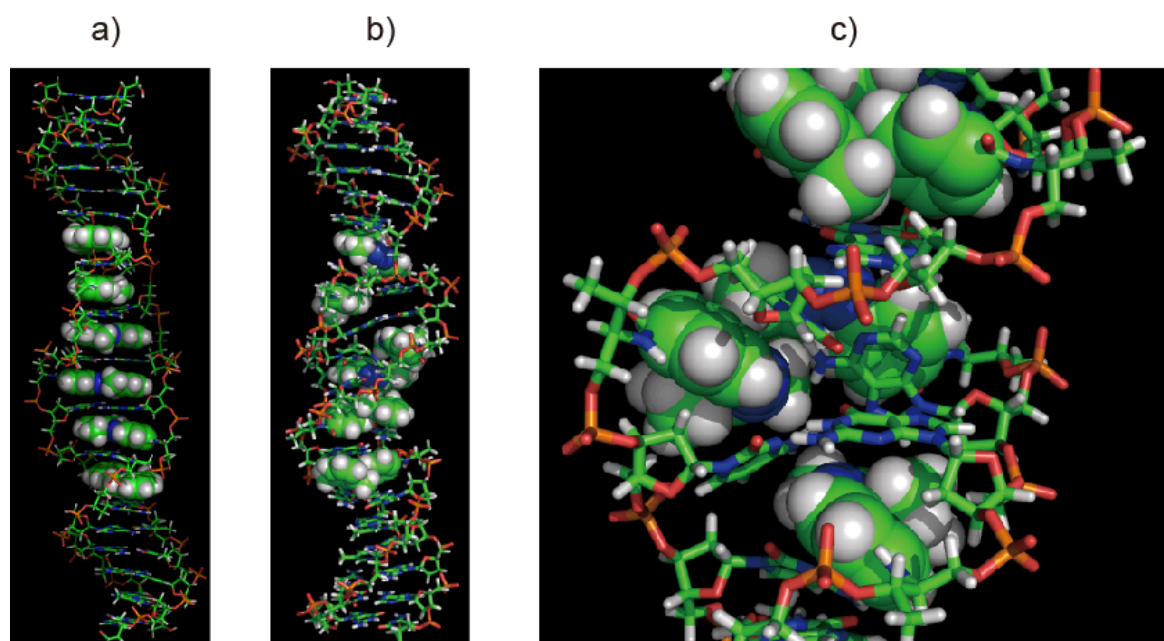


Supplemental Figure 3. T_m curves of duplex **XD1-T/XD2-A** obtained by monitoring the change of CD intensity with temperature for *trans* form at 350 nm. The T_m is 61.0°C, which is close to the T_m obtained by monitoring absorbance change with temperature at 260 nm. Conditions: 3.5 μ M DNA, 100 mM NaCl, pH 7.0 (10 mM phosphate buffer).

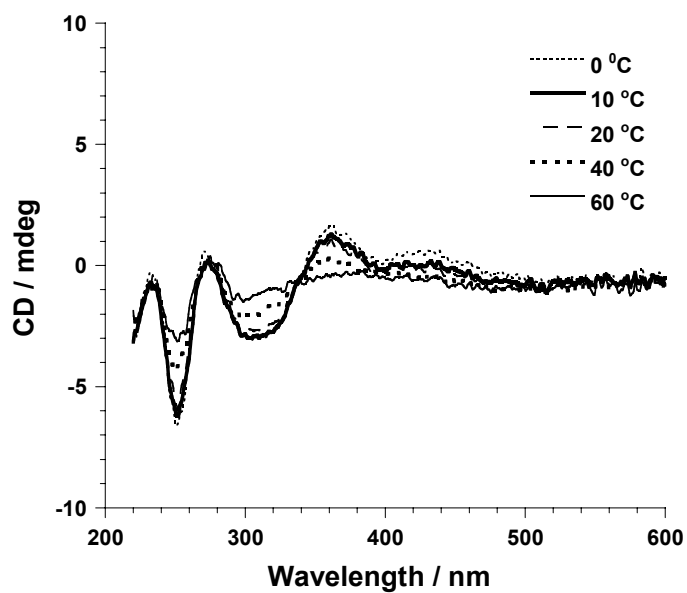


Supplemental Figure 4. T_m curves of duplex **XD3/XD4**. Conditions: 2.0 μM DNA, 100 mM NaCl, pH 7.0 (10 mM phosphate buffer).

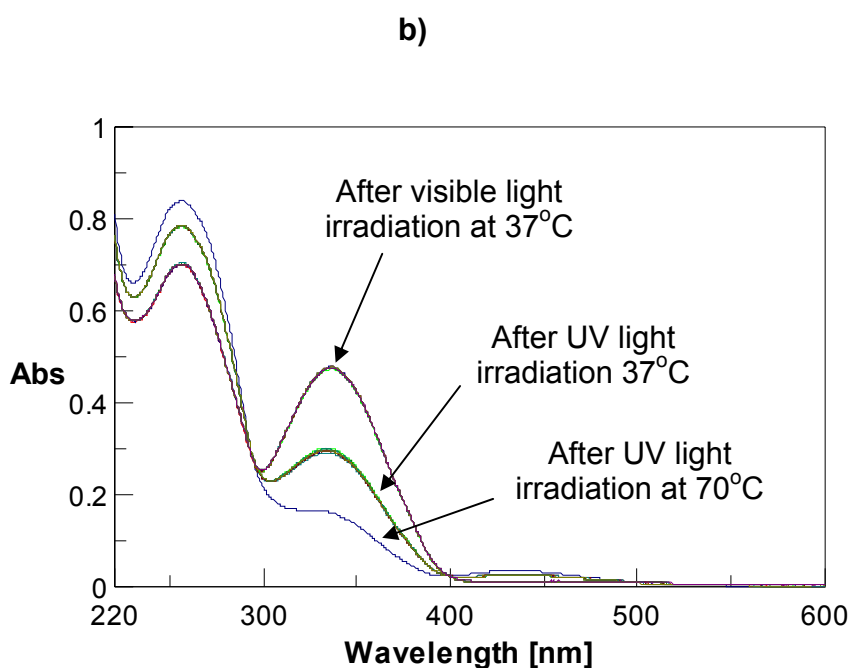
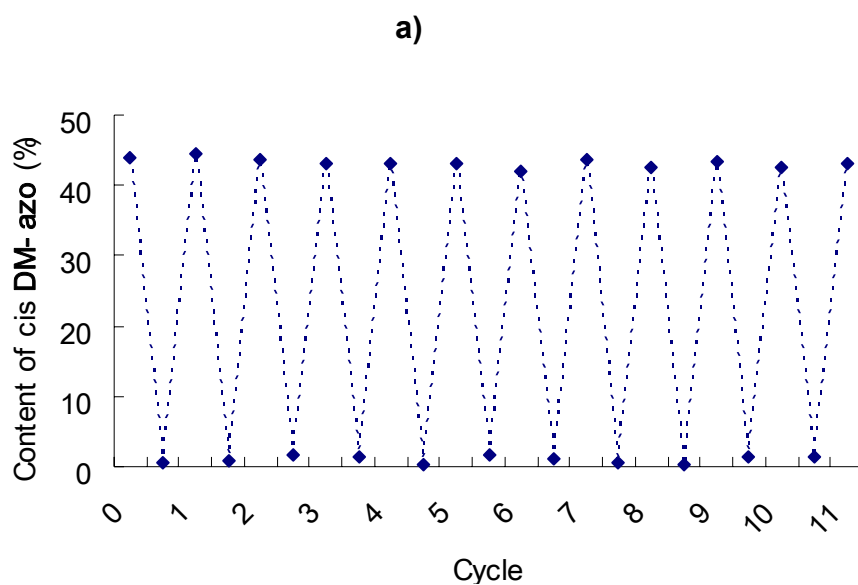
Melting temperature (T_m) of **nD3/nD4** duplex composed of natural 17 base pair was 63.2 °C. T_m s of **XD3/nD4** and **nD3/XD4** were determined to be 68.6 °C and 69.4 °C, respectively. This T_m increase was attributed to the stabilization effect of intercalated **DM-azos** which could *overcompensate* the distortion caused by the asymmetry. T_m of **XD3/XD4** containing the new duplex motif at the center was as large as 73.3 °C, which was 10.1 °C higher than that of native **nD3/nD4**.



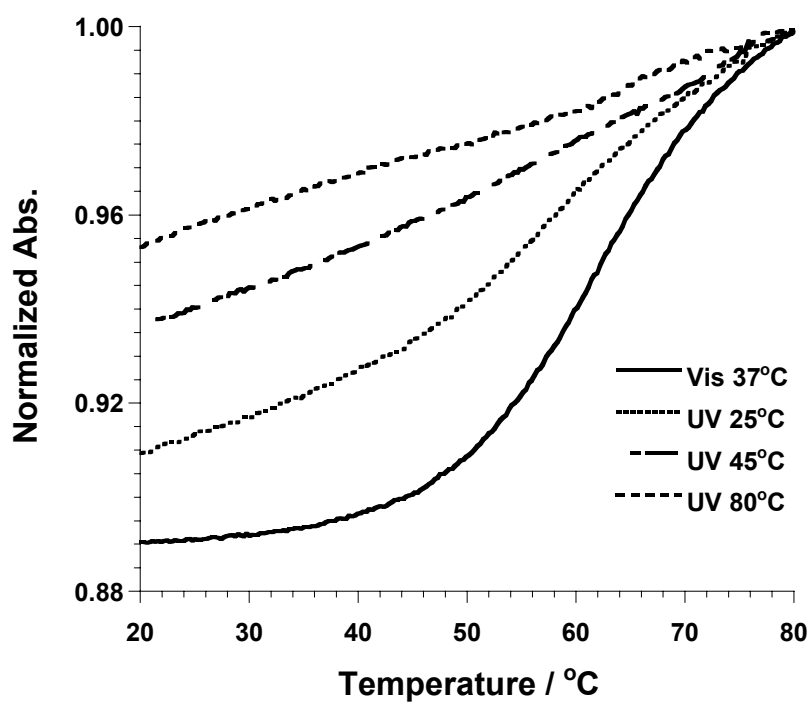
Supplemental Figure 5. Molecular modeling structure of duplex **XD3/XD4** involving 6 **DM-azo** moieties (a). **DM-azos** are highlighted in CPK (space-filling) model. The modeling structure of *cis* form is also shown in (b). The enlarged part involving *cis*-**DM-azos** base pairs are shown in (c).



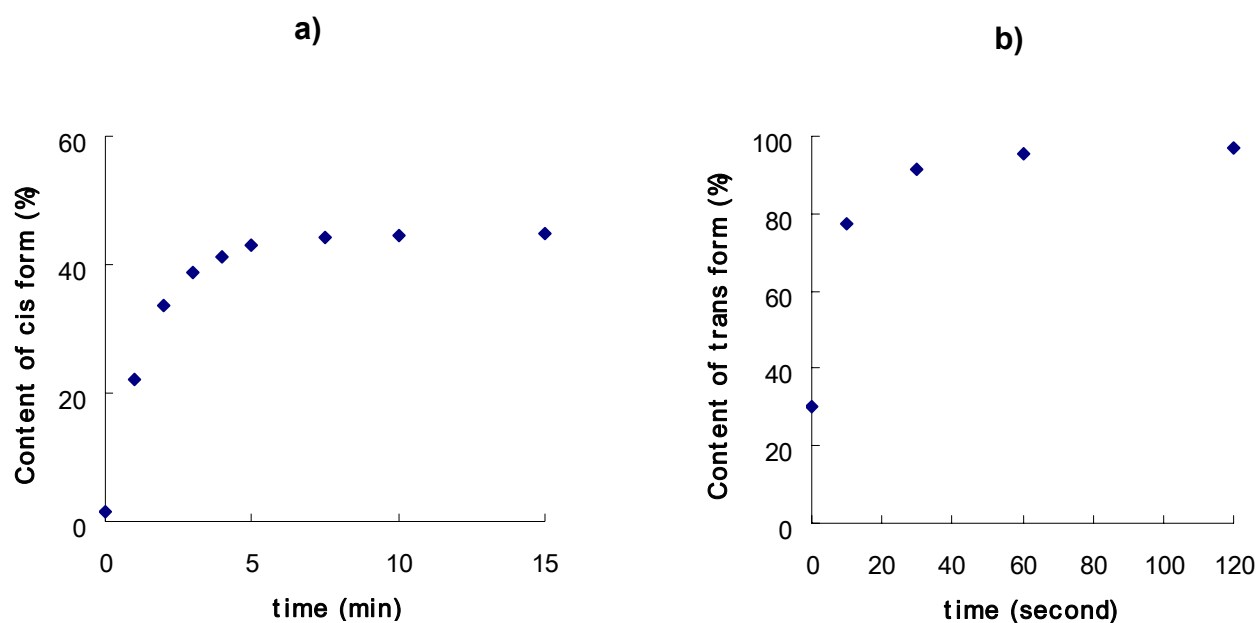
Supplemental Figure 6. CD spectra of *cis*-XD1-T/XD2-A at various temperatures. Conditions: 3.5 μ M DNA, 100 mM NaCl, pH 7.0 (10 mM phosphate buffer).



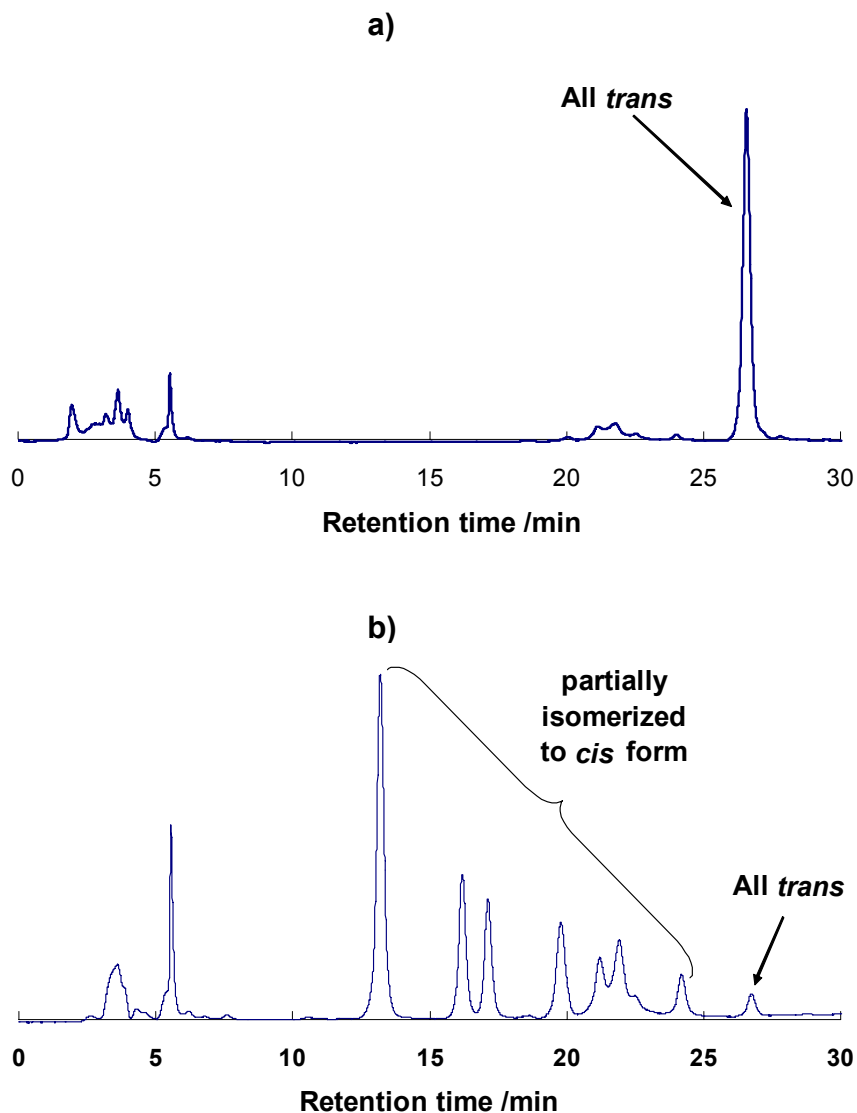
Supplemental Figure 7. Repetitive photoregulation of the formation of duplex **XD1-T/XD2-A** at 37°C. From the absorbance change at 260 nm, it can be deduced that formation and dissociation of the interstrand-wedged motif was successfully photoswitched by UV or visible light irradiation. The photoregulation efficiency was about 55% (96% duplexes formed under visible light and 59% duplex dissociated under UV light).



Supplemental Figure 8. T_m curves of XD1-T/XD2-A after UV irradiation at 70°C (dashed line), 45°C (dash-dotted line), 25°C (dotted line), and visible light irradiation at 37°C (solid line).



Supplemental Figure 9. Time course of the *trans*-to-*cis* photoisomerization of **DM-azos** in duplex **XD1-T/XD1-A** under UV light (a) and the *cis*-to-*trans* photoisomerization under visible light (b). The photoregulation was carried out at 37°C. 5 min of UV irradiation and 1 min of visible light irradiation are enough for the photoisomerization, respectively.



Supplemental Figure 10. HPLC chart of **XD1-C**. (a) The *trans* form obtained after incubation at 90°C for 6 h in the phosphate buffer (100 mM NaCl, pH 7.0); (b) The *cis* form obtained after UV light irradiation at 70°C. HPLC was carried out on a ODS column (Merck LiChrospher 100 RP-18(e)) at room temperature. A liner gradient of 20% to 35% acetonitrile/water (containing 50 mM ammonium formate) in 30 min with the elution rate of 0.5 mL/min was used. Because less than 70% of azobenzenes were isomerized to *cis* form, several peaks belonged to various isomers were observed.

Liang *et al.* Supplemental Figure 10.