

## Electronic Supplementary Information (ESI)

### Light-driven reversible strand displacement using glycerol azobenzene inserted DNA

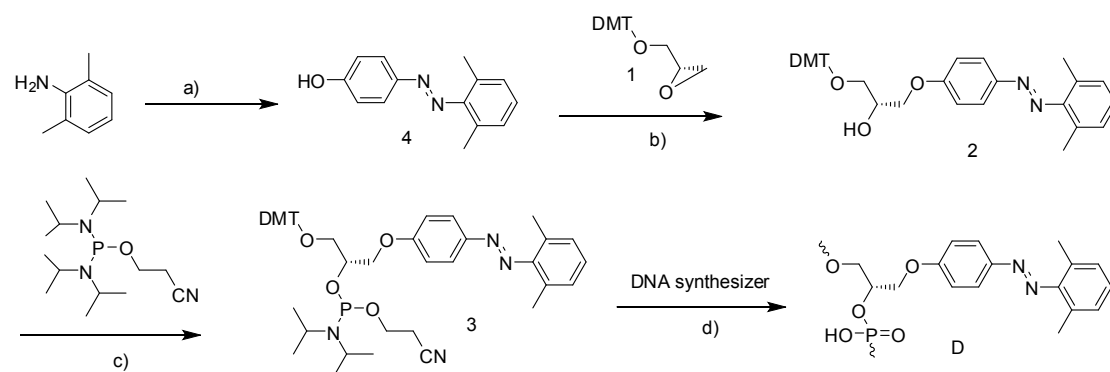
Bo Kou,<sup>ab</sup> Jiaxiao Zhang,<sup>b</sup> Xu Huai,<sup>b</sup> Xingguo Liang<sup>c</sup> and Shou-Jun. Xiao<sup>\*a</sup>

<sup>a</sup> State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing National Laboratory of Microstructures, Nanjing University, Nanjing 210093, China. E-mail: sjxiao@nju.edu.cn

<sup>b</sup> School of Materials Science and Engineering, Nanjing Institute of Technology, Nanjing 211167, China

<sup>c</sup> School of Food Science and Engineering, Ocean University of China, Qingdao 266003, PR China

#### Syntheses of DMazo-gDNA



**Scheme S1** Synthesis of DMazo-gDNA. Reagents and conditions: a)  $\text{NaNO}_2$ ,  $\text{HCl}$ ; and then  $\text{NaOH}$ , phenol,  $0\text{ }^\circ\text{C}$ , 10 min, 71 %; b) DMT-glycerol,  $\text{K}_2\text{CO}_3$ , DMF, reflux, 48 h, 54 %; c) 1H-tetrazole,  $\text{NC}(\text{CH}_2)_2\text{OP}(\text{N}(\text{iPr})_2)_2$ ,  $\text{CH}_3\text{CN}$ ,  $0\text{ }^\circ\text{C} \rightarrow \text{RT}$ , 1h, 83 %; d) DNA synthesizer.

**Synthesis of compound 4:** To a stirred and ice-cooled solution of 2, 6-dimethylaniline (1.5 ml, 12 mmol) and hydrochloric acid (20%, 6 ml, 36 mmol), sodium nitrite (0.9 g in 13 ml water, 13 mmol) was added, followed by phenol (1.13 g, 12 mmol) and  $\text{NaOH}$  (1.44 g in 30 ml water, 36 mmol). After stirring for 10 min, the solution was filtered. Excessive hydrochloric acid was added to the filtrate and the raw sediments were washed and dried. The sediments were dissolved in an alkaline solution and precipitated in an acidic solution 3 times to give a gray yellow powder (1.9 g, 71 % yield).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 7.85 (d, 2H; NN *aryl*), 7.11 (m, 3H; *aryl*), 6.92 (d, 2H; O *aryl*), 5.34 (m, 1H; OH), 2.30 (s, 6H;  $\text{CH}_3$ ). ESI-MS ( $\text{CH}_3\text{OH}$ )  $m/z$ :  $[\text{M}-\text{H}]^-$  calcd for  $\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}$ , 226.3; found, 225.3.

**Synthesis of compound 2:** A mixture of DMT-glycerol (compound 1 in our previous article,<sup>1</sup> 3 g, 8 mmol), compound 4 (1.8 g, 8 mmol) and  $\text{K}_2\text{CO}_3$  (14.4 g) in dimethylformamide (DMF, 60 mL) was refluxed for 48 h. After evaporation of DMF, the oily residue was dissolved in  $\text{CHCl}_3$ , washed with 100 ml saturated aqueous solution of  $\text{NaHCO}_3$  and subsequently  $\text{NaCl}$ , the organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated. The crude product was purified by a silica column chromatography, using petroleum ether/ethyl acetate (80: 20) as the eluant ( $R_f =$

0.5), to afford an orange solid (2.6 g, 54 % yield). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, δ): 7.88 (m, 2H; O *aryl* NN), 7.43 (m, 2H; C *aryl*), 7.32 (m, 4H; C *aryl* OCH<sub>3</sub>), 7.29 (m, 2H; C *aryl*), 7.17 (m, 1H; C *aryl*), 7.11 (m, 3H; NN *aryl*), 7.00 (m, 2H; O *aryl* NN), 6.82 (m, 4H; C *aryl* OCH<sub>3</sub>), 4.16 (m, 1H; CH), 4.14 (m, 2H; CH<sub>2</sub>O-azobenzene), 3.78 (s, 6H; OCH<sub>3</sub>), 3.37 (m, 2H; CH<sub>2</sub>O-DMT), 2.31 (s, 6H; aryl-CH<sub>3</sub>), 1.92 (d, 1H; OH). ESI-MS (CH<sub>3</sub>OH) m/z: [M+Na]<sup>+</sup> calcd for C<sub>38</sub>H<sub>38</sub>N<sub>2</sub>O<sub>5</sub>, 625.7; found, 625.5.

**Synthesis of compound 3:** 2-Cyanoethyl N,N,N',N'-tetraisopropylphosphordiamidite (0.8 ml, 0.76 g, 2.5 mmol) was added into 40 ml dry CH<sub>3</sub>CN solution containing compound 2 (1.1 g, 1.8 mmol) at 0 °C, cooled by an external ice bath. Then the 1H-tetrazole solution (0.15 g dry 1H-tetrazole dissolved in 20 ml dry CH<sub>3</sub>CN) was added drop-wise. After approximately 1 h stirring at room temperature under dry nitrogen, the solvent was evaporated, and the residual oily product was dissolved in ethyl acetate. Washed with 100 ml saturated aqueous solution of NaHCO<sub>3</sub> and subsequently NaCl, the organic phase was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> (20 g), filtered, and concentrated. The crude oily product of compound 3 was purified by a silica column chromatography (petroleum ether/ethyl acetate/triethylamine 75:25:2 as the eluant, R<sub>f</sub>=0.63) to afford a yellow-orange foamy solid (1.2 g, 83% yield). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, δ): 7.88 (m, 2H; O *aryl* NN), 7.45 (m, 2H; C *aryl*), 7.33 (m, 4H; C *aryl* OCH<sub>3</sub>), 7.27 (m, 2H; C *aryl*), 7.21 (m, 1H; C *aryl*), 7.10 (m, 3H; NN *aryl*), 7.01 (m, 2H; O *aryl* NN), 6.81 (m, 4H; C *aryl* OCH<sub>3</sub>), 4.35 (m, 1H; CHOP), 4.22 and 4.12 (m, 2H; C H<sub>2</sub>O-azobenzene), 3.77 (s, 6H; OCH<sub>3</sub>), 3.65-3.54 (m, 2H; CH(CH<sub>3</sub>)<sub>2</sub>), 3.40 (m, 2H; CH<sub>2</sub>O-DMT), 3.34-3.28 (m, 2H; CH<sub>2</sub>OP), 2.57-2.47 (m, 2H; CH<sub>2</sub>CN), 2.31 (s, 6H; aryl-CH<sub>3</sub>), 1.20-1.13 (m, 12H; CH(CH<sub>3</sub>)<sub>2</sub>). ESI-MS (CH<sub>3</sub>OH) m/z: [M+Na]<sup>+</sup> calcd for C<sub>47</sub>H<sub>55</sub>N<sub>4</sub>O<sub>6</sub>P, 825.9; found 825.8.

**Synthesis of azobenzene-inserted DNA:** All modified DNAs used in this study were synthesized by Shanghai Generay Biotech Co. Ltd. with the phosphoramidite monomers of glycerol-tethered 2', 6'-dimethylazobenzene (Compound 3) and conventional monomers, and purified by PAGE. The coupling step for azobenzene-carried phosphordiamidite (compound 3) generally takes 600 s. MALDI-TOF-MS m/z: D16, 16270 (calcd, 16266); D16D, 17289 (calcd, 17285).

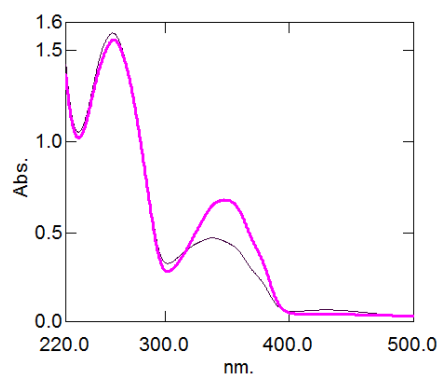
### Photoisomerization of azobenzene-inserted DNA

The light source for the photo irradiation was a 120 W alternative light source (Portable multi-band light source, FGG-II, Beijing Jiatai Yu Tong Police Gear and Equipment Technology Co., Ltd.). The sample solution was added to a quartz cell and the temperature of light irradiation was set by a programmable temperature controller (S1700, Shimadzu). The light irradiation was taken in a dark room. For the *trans*-to-*cis* isomerization, the DNA solution was irradiated by a UV light at 365 nm, while the *cis*-to-*trans* isomerization was carried out by irradiation with a visible light at 475 nm. For the UV-visible absorption spectra, UV or visible light irradiation lasted for 2 min, while for the fluorescent measurements irradiation only lasted for 20 s.

### UV-visible absorption spectra

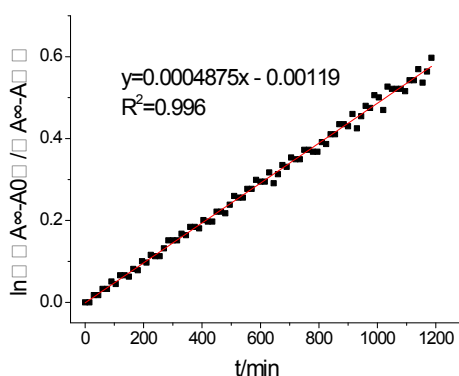
UV-visible absorption spectra of the azobenzene-inserted DNA solution (2 μM DNA, 150 mM NaCl, 10 mM phosphate buffer at pH 7.0) were measured by a UV-visible spectrometer (UV-1800,

Shimadzu) equipped with S1700.



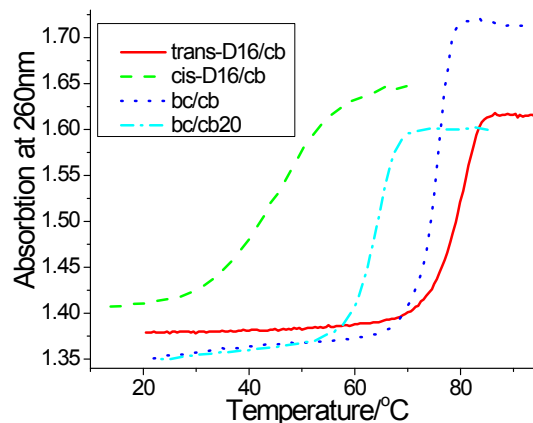
**Fig. S1** UV-visible absorption spectra of D16/bc after irradiation by visible (thick line) and UV (thin line) light at 25 °C. D16: 5'-CCDCTCADTTDCADATDACCCDTADCGDTCDCADTGDTCDACDTTC-3', bc: 5'-GAAGTGACATGGAGACGTAGGGTATTGAATGAGGG-3'.

**Half-life measurement of *cis*-to-*trans* thermal isomerization of DMazo-gDNA.** Details for half-life time measurements can be found in Supporting Information of our previous article.<sup>1</sup> Briefly, the decay rate coefficient and half-life time can be calculated by the absorbance at 340 nm of D16/Fbc at the initial point ( $A_{340}^0$ ) and at a particular time ( $A_{340}^t$ ), and the absorbance of the completely decayed *trans*-D16/Fbc ( $A_{340}^\infty$ ). The decay rate coefficient of  $k$  can be obtained as the slope of the linear plot of  $\ln((A_{340}^\infty - A_{340}^0) / (A_{340}^\infty - A_{340}^t))$  against  $t$  as  $\ln((A_{340}^\infty - A_{340}^0) / (A_{340}^\infty - A_{340}^t)) = kt$ , then the half-life time can be calculated as:  $t_{1/2} = \ln 2 / k = \ln 2 / 0.0004875 = 1422$  min (~24 h).



**Fig. S2** The linear plot of  $\ln((A_{350}^\infty - A_{350}^0) / (A_{350}^\infty - A_{350}^t))$  as a function of time.

**$T_m$  measurement.** The  $T_m$  values in Fig. S2 were determined from the maximum in the first derivative of the melting curve, which was obtained by measuring the absorbance at 260 nm as a function of temperature. The temperature ramp was 1.0 °C/min.



**Fig. S3** Melting curves of the native and DMazo modified 35-nt-long DNA duplex.

Solution conditions: 2  $\mu$ M DNA, 150 mM NaCl, 10 mM phosphate buffer at pH 7.0

D16: 5'-CCDCTDCADTTDCADATDACDCCDTADCGDTCDCADTGDTCDACDTTC-3'

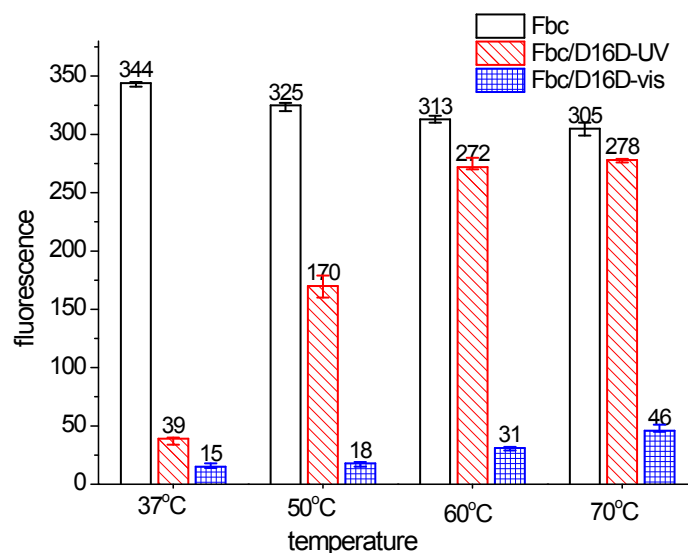
cb: 5'-GAAGTGACATGGAGACGTAGGGTATTGAATGAGGG-3'

bc: 5'-CCCTCATCAATACCCTACGTCTCCATGTCACTTC-3'

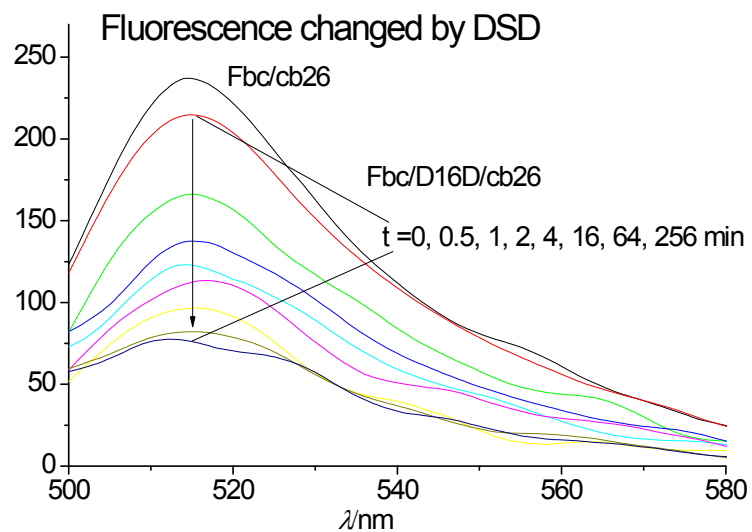
cb20: 5'-CGTAGGGTATTGAATGAGGG-3'

## Fluorescence measurement

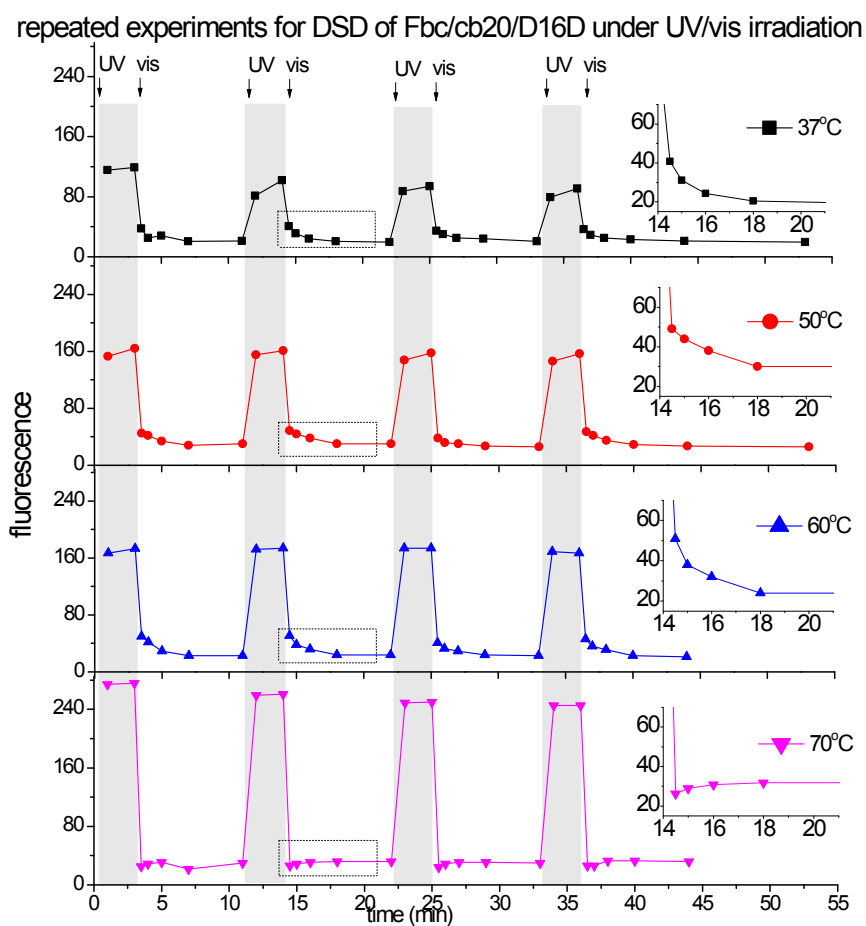
Fluorescence spectra of Fbc excited at 490 nm was measured with a fluorescence spectrophotometer (LS-55, PerkinElmer). The association fraction of duplex Fbc/D16D was calculated by the amount of quenched FAM fluorescence emission at 515 nm with respect to the single stranded Fbc at the same concentration. The same calculation approach was applied to the DNA strand displacement system of Fbc/cb20/D16D, while the control fluorescence intensity counted on the duplex of Fbc/cb20-29 at the same concentration. In any case, more than 4 cycles of the alternative UV/visible light irradiation were carried out to ensure the facticity.



**Fig. S4** Photoregulated hybridization demonstrated by the fluorescence emission at 515 nm. The numerical values above error bars are averaged from 4 execution cycles. Solution conditions: 100 nM Fbc or cb, 140 nM D16D, 150 mM NaCl, 10 mM phosphate buffer at pH 7.0.



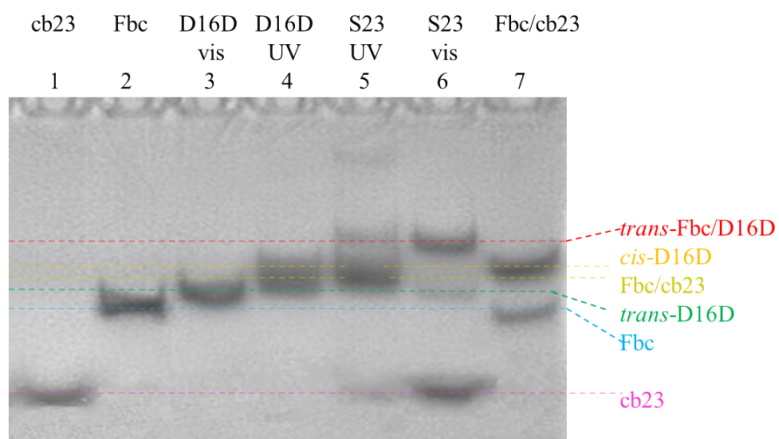
**Fig. S5** Evolution of fluorescence spectra against time in the DSD system of Fbc/D16D/cb26 at 50 °C. Solution conditions: 100 nM Fbc, 120 nM cb26, 140 nM D16D, 150 mM NaCl, 10 mM phosphate buffer at pH 7.0.



**Fig. S6** Reversible light-driven DSD at different temperatures demonstrated by fluorescence changes. The insert graph showed the enlarged dash square. Solution conditions: 100 nM Fbc, 120 nM cb20 140 nM D16D, 150 mM NaCl, 10 mM phosphate buffer at pH 7.0.

## Non-denaturing PAGE gel electrophoresis

Electrophoresis was run on a 1.5 mm thick non-denaturing PAGE gel using a constant voltage of 100 V for 90 minutes. After electrophoresis, the gels were stained with Stains-All for 30 min and imaged as shown in Fig. 5 and Fig. S7. UV and visible light irradiation was carried out at 50 °C for 2 min before loading.



**Fig. S7** The light-driven DSD of Fbc/D16D/cb23 (S23, Fbc : D16D : cb23= 1:1:1) proved by electrophoresis on an 18% non-denaturing PAGE gel (TBE buffer). UV and visible light irradiation was carried out at 50 °C for 2 min before loading.

## References

1. B. Kou, X. Guo, S. Xiao and X. Liang, *Small*, 2013, **9**, 3939-3943.