Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Single-Molecule Photon-Fueled DNA Nanoscissors for DNA Cleavage based on the Regulation of Substrate Binding Affinity by Azobenzene

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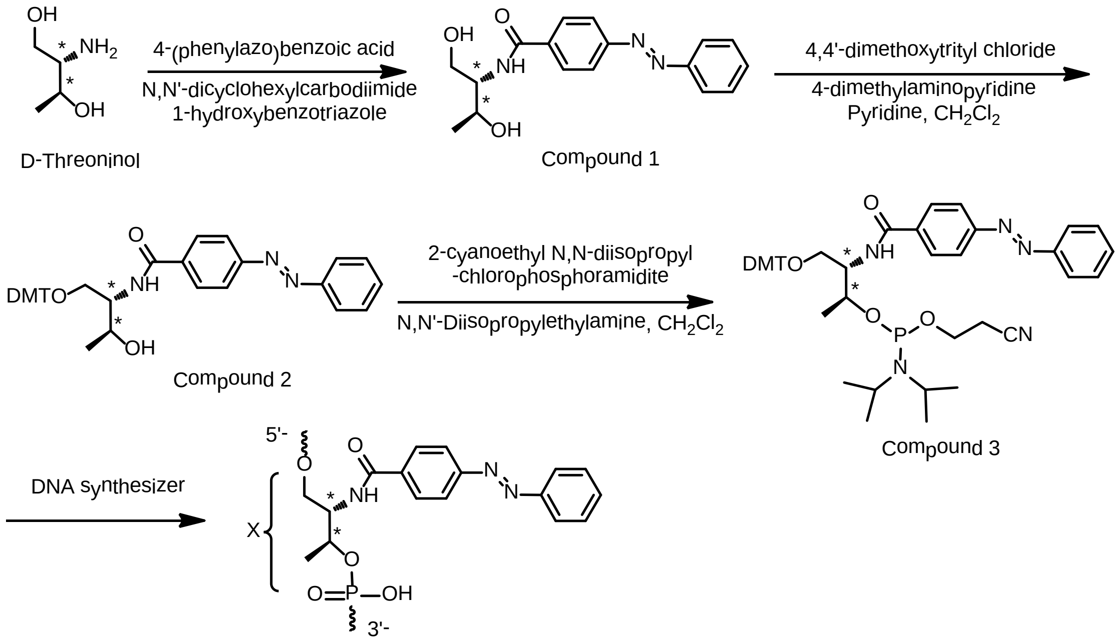
1. Materials.

All the DNA synthesis reagents and 4,4’-dimethoxytrityl chloride were purchased from Sigma–Proligo (Germany). D-threoninol, 4-(phenylazo)benzoic acid, dicyclohexylcarbodiimide, hydroxybenzotriazole, 4-dimethylaminopyrid-ine, N, N'-diisopropylethylamine were purchased from Sigma (Shanghai, China). Other reagents were purchased from Sinopharm Chemical Reagents (Shanghai, China).

2. Synthesis of azobenzene tethered phosphoramidite monomer



Compound 1: In a 10 ml round-bottomed flask, a solution of D-threoninol (100mg, 0.95 mmol), 4-(phenylazo)benzoic acid (238 mg, 1.05 mmol), dicyclohexylcarbodiimide (DCC) (217 mg, 1.05 mmol) and hydroxybenzotriazole (HOBt) (142 mg, 1.05 mmol) in 5.3 ml dry DMF was stirred under a nitrogen atmosphere at room temperature. The reaction was checked by TLC with 7:3 (vol/vol) ethyl acetate/petroleum ether mobile phase. The reaction mixture was filtered and then a 5-fold volume of water was added, followed by a one-fold volume of diethyl ether to extract the product. The extraction was repeated for a total of five times. The organic phase was washed with saturated $NaHCO_3$ solution and saturated NaCl solution and dried over anhydrous $MgSO_4$. The solvent was removed with a rotary vacuum evaporator, and the residue was purified by column chromatography (ethyl acetate/methanol: 25/1) and dried to afford compound 1 (202

mg, 0.65 mmol, 68%) as an orange solid. ^{1}H NMR: (400 MHz, $CDCl_3$): δ7.90-8.00(m, 6H) , δ 7.45-7.55(m, 3H), δ 7.03-7.08 (d, 1H), δ 4.29-4.35(m, 1H), δ 4.05-4.10(m, 1H), δ 3.95-4.00 (d, 2H), δ 1.25-1.32(d, 3H). ESI-MS (m/z) $[1+H]^+$ calcd 314.1, obsd 314.8.

Compound 2: Compound 1 (100 mg, 0.32 mmol) and 4-dimethylaminopyridine (1.95 mg, 0.016 mmol) were dissolved in 1.80 mLdry pyridine in a 10 mL round-bottomed flask under dry nitrogen. In a separate 10 mL round-bottomed flask, 4,4'-dimethoxytrityl chloride (338.83 mg, 0.384 mmol) was dissolved in 0.5 ml dry CH_2Cl_2 under nitrogen, and this solution was added to the above pyridine solution slowly under dry nitrogen on an ice bath with stirring. The mixture was stirred for 1h at 0 °C and then at room temperature for another 24h. The reaction was checked by TLC with 30:70:3 (vol/vol/vol) ethyl acetate/petroleum ether/triethylamine. The solvent (pyridine and CH_2Cl_2) was removed using a rotary evaporator, and the residue was purified by column chromatography (eluent: 30:70:3 (vol/vol/vol) ethyl acetate/petroleum ether/triethylamine) and dried to afford compound 2 (167 mg, 0.27 mmol, 85%) as an orange-red solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95-6.85(m,23H), δ 4.20-4.30(m, 1H), δ 4.10-4.20(m, 1H), δ 3.65-3.80(s, 6H), δ 3.57-3.63, δ 3.38-3.43(dd, 2H), δ 1.20-1.30 (d, 3H). ESI-MS (m/z) $[1+H]^+$ calcd 616.3, obsd 615.8.

Compound 3: To a solution containing compound 2 (100 mg, 0.16 mmol) in anhydrous CH_2Cl_2 (0.59 mL) at 0 °C, N, N'-diisopropylethylamine (DIPEA) (56.9 mg, 30.0 mmol) was added slowly under nitrogen. Then, 2-cyanoethyl diisopropyl chlorophosphoramidite (42 uL, 13 mmol) was added dropwise, and the reaction mixture was stirred at 0 °C for 1 h. The reaction was checked by TLC with 40:60:3 (vol/vol/vol) ethyl acetate/petroleum ether/triethylamine. After removing the solvent, the residue was dissolved in ethyl acetate, and the organic phase was washed with saturated $NaHCO_3$ solution and NaCl solution and dried over anhydrous $MgSO_{4.}$ The solvent was evaporated, and the residue was purified by column chromatography and dried to afford compound 3 (98 mg, 0.12 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00-6.79 (m, 22H), δ 6.62 (d, 1H), δ 4.48 (m,1H), δ 4.39 (m, 1H), δ 4.21-4.10 (m, 2H), δ 3.76-3.77 (s, 6H), δ 3.57-3.34 (m, 4H), δ 2.76-2.72 (m, 2H), δ 1.30-1.25 (m, 15H). ^{31}P ($CDCl_3$): δ 149. ESI-MS (m/z) $[1+H]^+$ calcd 816.4, obsd 816.0.

3. DNA Synthesis.

All the oligonucleotides used in the work were synthesized in house. The DNA product was synthesized on a 12-Column DNA Synthesizer (PolyGen GmbH). Azobenzene groups were incorporated using azobenzene tethered phosphoramidite monomer. The synthesis protocol was set up according to the requirements specified by the reagents' manufacturers. Following machine synthesis, the DNA product was deprotected and cleaved from CPG by incubating with 0.4 mL ammonium hydroxide for 8 hours at 65°C in a water bath. The cleaved DNA product was precipitated in 40 µL 3.0 M NaCl and 1.2 mL ethanol. After the supernatant was removed, the precipitated DNA product was dissolved in 500 µL 0.1 M triethylamine acetate (TEAA) and purified by reverse phase HPLC using a C18 column with 100 mM TEAA, pH 7.5 and acetonitrile (0–4min, 0-0%; 4-30 min, 10-65%) as an eluent on an Agilent 1100 HPLC. The collected DNA product was dried and detritylated by incubating in 200 µL 80% acetic acid for 20 minutes. The detritylated DNA product was mixed with 400 µL ethanol and dried by a vacuum dryer. The purified probe was quantified by determining the UV absorption at 260 nm, after which the probe was dissolved in DI water and stored at -20°C for future experiments.

Table S1. Sequences of oligonucleotides synthesized.

Oligo	*Sequence(5'-3')*
A3R7Enz35	GXC TXT AXC CTT TTT TTG GTA AGC CTG GGC CTC TTT CTT TTT AAG AAA GAA C
R8Enz35	GGC TTAC CTT TTG GTA AGC CTG GGC CTC TTT CTT TTT AAG AAA GAA C
R7Enz35	GCT TACC TTT TGG TAA GCC TGG GCC TCT TTC TTT TTA AGA AAG AAC
R6Enz35	CTT ACC TTT TGG TAA GCC TGG GCC TCT TTC TTT TTA AGA AAG AAC
R5Enz35	TTA CCT TTT GGT AAG CCT GGG CCT CTT TCT TTT TAA GAA AGA AC
R4Enz35	TAC CTT TTG GTA AGC CTG GGC CTC TTT CTT TTT AAG AAA GAA C
Enz35	GGT AAG CCT GGG CCT CTT TCT TTT TAA GAA AGA AC
Sub24	F-AGC TTC TTT CTA ATA CGG CTT ACC-Q
H2	GXC TXT AXC CTT TTT TTG GTA AGC C

F stands for fluorescein. Q stands for DABCYL. X stands for azobenzene.

4. DNA digestion by DNA nanoscissors or DNAzyme.

Typical procedure for DNA digestion by the DNA nanoscissors or DNAzyme was as follows. First, either visible light (table lamp, GD-8024, 11W) or UV light (UV lamp, SB100-P/F,4.0 mW/cm-2) irradiation was applied to the DNAzyme. Then, the irradiated DNAzyme (50 nM) was added to a buffer (1.5 M NaCl, 50 mM Tris-HCl, pH 7.5) containing 50 nM Sub24. Then, 20 μM Cu^{2+} and 20 μM H_2O_2 were added to initiate the cleavage reaction, and the reaction mixture was incubated for 1h at room temperature. For the cycled DNA digestion, UV and visible light were applied alternately for 2 min and 5 min, and then DNAzyme was added to the digestion solution.

5. The length optimization of regulatory domain

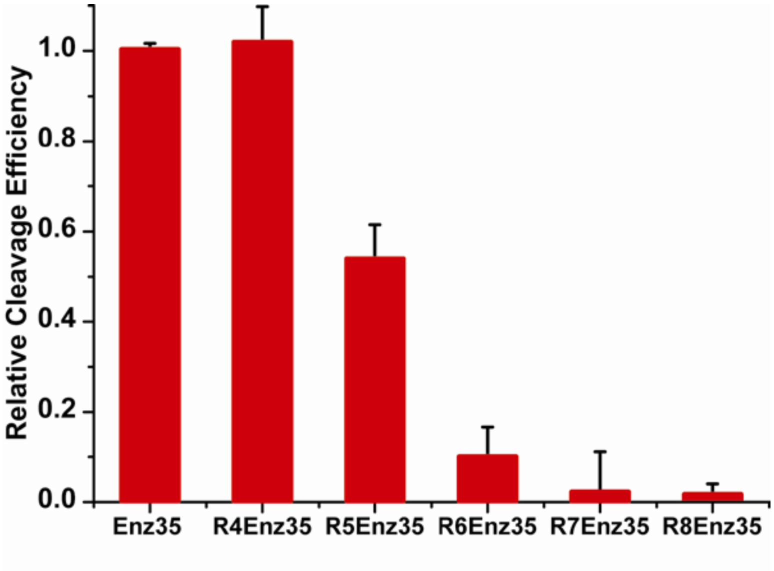


Figure S1. Inhibition of DNAzyme activity with regulatory domain of different lengths. Different lengths (4, 5, 6, 7, and 8 bases) of regulatory domain complementary to the DNAzyme were added to 5’-end of the DNAzyme, yielding R4Enz35, R5Enz35, R6Enz35, R7Enz35 and R8Enz35, respectively.

6. Melting Temperature of regulatory domain.

The melting temperature (T_m) of the stem duplex part (Hairpin H2) involving alternating azobenzene moieties and base pairs was measured in PBS with 300 mM NaCl. Melting profiles were monitored at 260 nm on a UV spectrophotometer (Agilent 8453) equipped with a Peltier temperature controller. The melting profile was monitored starting from 25°C using a heating rate of 2.5°C/min.

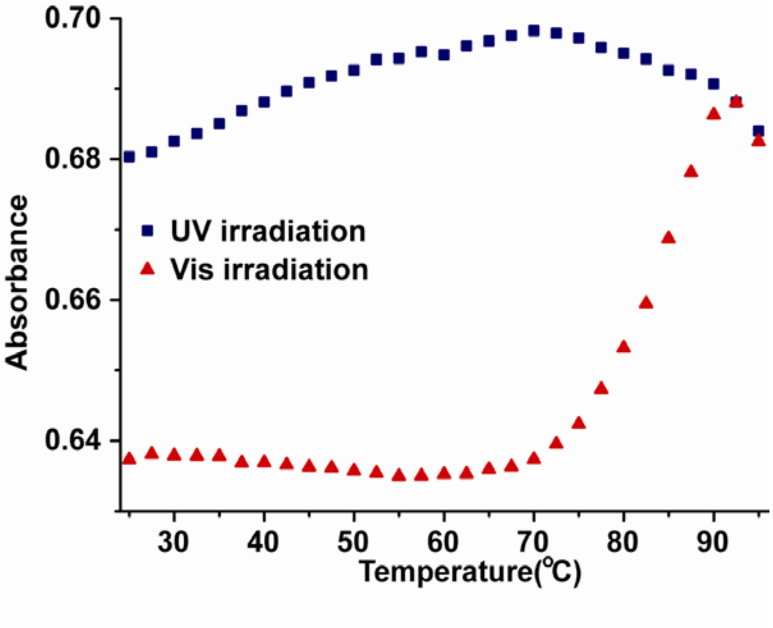


Figure S2. The melting temperature (T_m) of the hairpin **H2** with 3 alternating azobenzene moieties (**H2**: 5’-G*X*C T*X*T A*X*C CTT TTT TTG GTA AGC C, *X* stands for azobenzene).

7. HPLC analysis of *trans* to *cis* photoswitching efficiency following UV irradiation

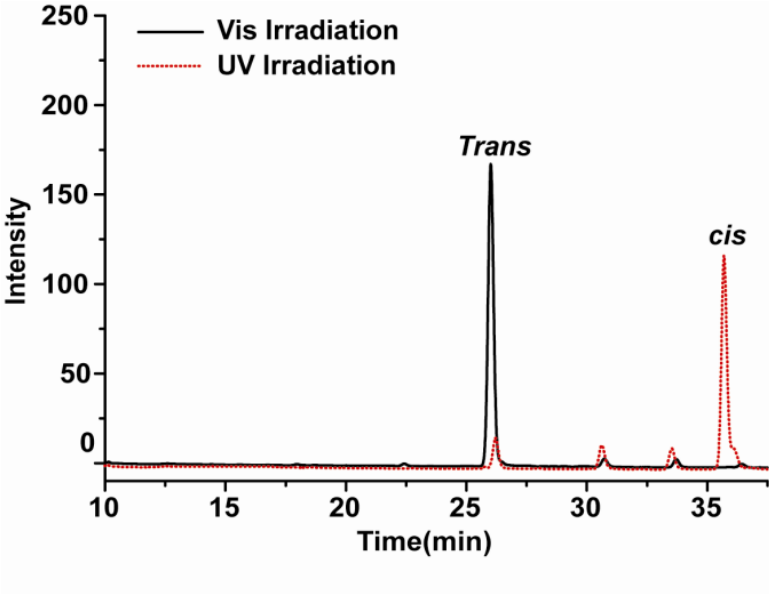


Figure S3. HPLC analysis of *trans* to *cis* photoswitching efficiency of A3R7Enz35 following UV irradiation. Analysis of A3R7Enz35 irradiated with either UV or visible light was performed by reverse phase HPLC using a C18 column with 100 mM triethylamine-acetic acid buffer (TEAA, pH 7.5) and acetonitrile (0–3 min, 0-0%; 3-53 min, 10-25%) as an eluent on an Agilent 1100 HPLC.

8. **Real time fluorescence monitoring of the DNA nanoscissors**

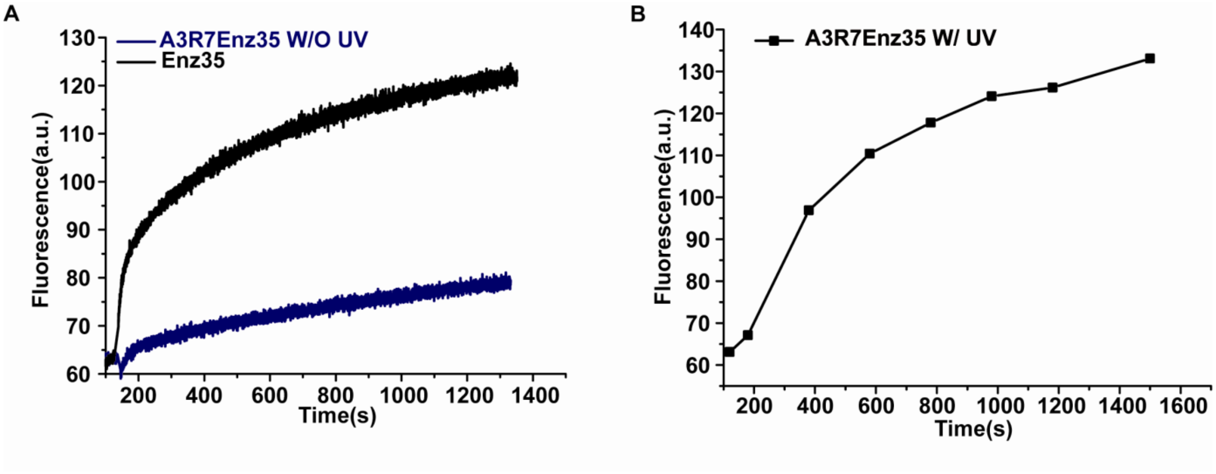


Figure S4. Time course study of the DNA Nanoscissors before UV irradiation (A) and after UV irradiation (B). Because the azobenzene groups will transform from the *trans* form to the *cis* form under visible light, the active nanoscissors A3R7Enz35 would become a close state slowly under the fluorescent light source (490nm). The fluorescence of the nanoscissors after UV irradiation is detected every 3 min.

9. Kinetics study of photoswitching efficiency by measuring the absorbance after UV irradiation

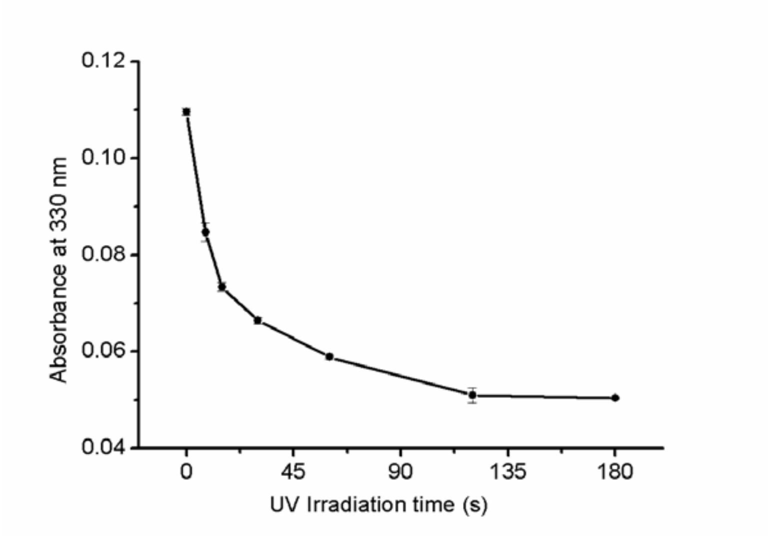


Figure S5. Kinetics study of photoswitching efficiency. A solution of A3R7Enz35 was irradiated with UV light for different times, followed by absorbance measurement at 330 nm, the λ_{max} of the π-π* transition of *trans*-azobenzene .