Structural stability of the photo-responsive DNA duplexes containing one azobenzene via a confined pore

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Supplementary Information

1. Materials.

All aqueous solutions for analytical studies were prepared with ultrapure water (reaching a resistivity of 18.2 MΩ·cm at 25 °C) from the Milli-Q System (EMD Millipore, Billerica, MA, U.S.A.). The information of reagents was as follows: decane (Sigma-Aldrich, St. Louis, MO, USA), 1,2-diphytanoly-sn-glycero-3-phosphocholine (Avanti Polar Lipids Inc, Alabaster, AL, USA). Wild-type *α*-HL was produced as described in our previous studies.[1] The azobenzene-modified single-stranded DNA (azo-ssDNA) was obtained by tethering the azobenzene moiety to phosphate linkage along the DNA backbone via a D-threoninol group.[2] The azobenzene group intercalates between adjacent base pairs in the duplex.[3-5] In the *trans*-form, the intercalated planar azobenzene enhanced a stacking interaction to stabilize the duplex (Fig. S1). Following UV irradiation, the non-planar *cis*-azobenzene was hypothesized to be a diminished ability to intercalate, leading to destabilization of the duplex.

Fig. S1 The duplex with planar *trans*-azobenzene intercalated. A azobenzene group was introduced into position X through a D-threoninol group.

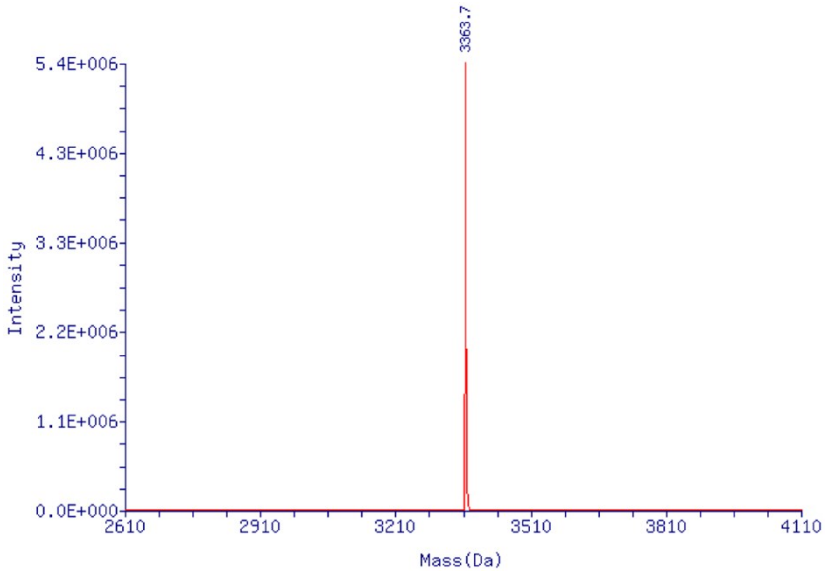


Fig. S2 HPLC-MS spectrum of azo-ssDNA. The calculated molecular weight is 3363.34 Da.

All the single-stranded DNA (ssDNA) were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The

HPLC-MS for azobenzene-modified ssDNA was shown in Fig. S2. The DNA duplexes were prepared by mixing the complementary single strands in a 1:1 ratio at a concentration of 50 μM in 0.66 M KCl, 10 mM Tris and 1 mM EDTA (pH 8.0), followed by heating the sample to 100 °C, then slowly cooling to room temperature. All the other chemicals were of analytical grade unless otherwise indicated.

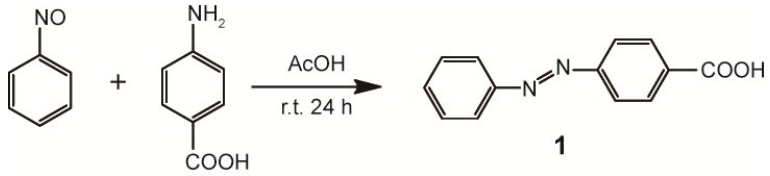
2. Nanopore Formation and Data Collection.

The details of the nanopore formation are described in our previous research.[1] The two chambers beside the nanopore were filled with electrolyte solution (1 M KCl, 10 mM Tris and 1 mM EDTA at pH 8.0). The experiments were conducted using eONE amplifier (Elements SRL, Cesena, Italy). The sampling rate was set to 100 kHz and the final bandwidth 5 kHz. The data were acquired by EDR 3 software (Elements SRL, Cesena, Italy). Data analysis was performed using MOSAIC software[6] and Origin 8.0 (OriginLab Corporation, Northampton, MA, USA). The unzipping times of the blockage events were fitted by exponential function. The event frequencies were obtained by plotting the relation curves between cumulative event count versus recording time.

3. Synthesis the azobenzene-tethered compound for incorporating into DNA

The Compound 4, a phosphoramidite compound bearing an azobenzene functionality covalently connected through an amide bond, was synthesized according to the following four steps.[2] Then Compound 4 can be incorporated into DNA sequence by Sangon Biothech. Co. Ltd.

Step 1:



Nitrobenzene (1.00 g, 9.34 mmol) and p-aminobenzoic acid (1.54 g, 11.23 mmol) were suspended in glacial acetic (15 ml). Then the mixture was stirring at room temperature for 24 h. The suspension was filtered, washed by water and then purified by recrystallization from ethyl acetate to give pure Compound 1 as an orange solid (1.64 g, 7.26 mmol 78% yield) ^{1}H NMR (DMSO); δ=13.1 (s, brs, 1H, -COO<u>H</u>), 7.56-7.65 (m, 3H, ArH), 7.88-7.99 (m, 4H, ArH), 8.10-8.17 (m, 2H, ArH). ^{13}C NMR (101 MHz, DMSO) δ 175.82, 152.62, 152.47, 143.30, 130.50, 129.97, 122.97, 122.09. HRMS (ESI, *m/z*): calcd for $C_{13}H_9N_2O_2$ (M^-) 225.0664, found mass 225.0660.

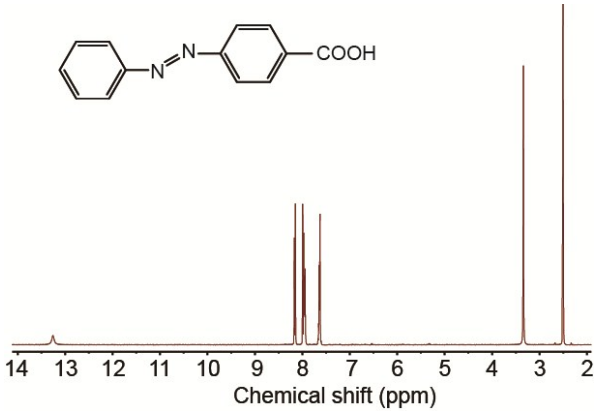


Fig. S3 ^{1}H NMR spectroscopy (400 MHz) in DMSO of Compound 1.

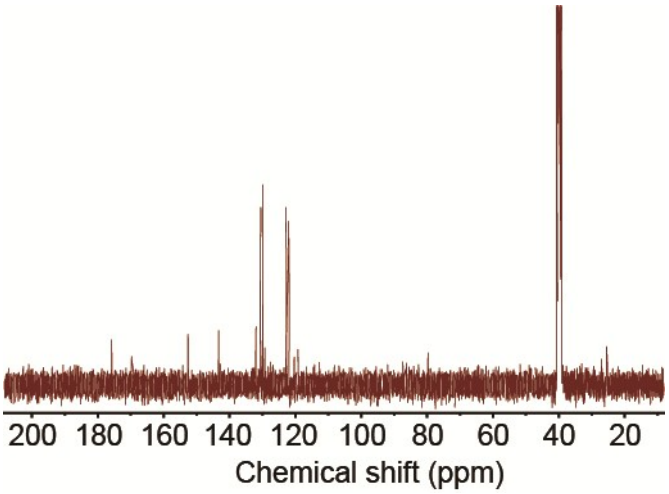
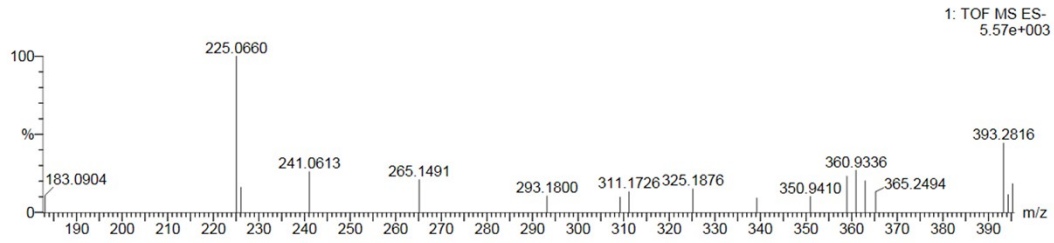


Fig. S4 ^{13}C NMR spectroscopy (101 MHz) in $CDCl_3$ of Compound 1.

Elemental Composition Report

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

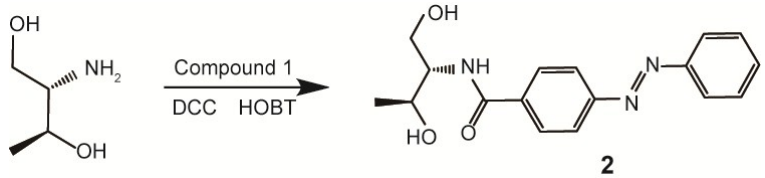
Monoisotopic Mass, Even Electron Ions
34 formula(e) evaluated with 3 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-26 H: 0-24 N: 0-3 O: 0-3



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
225.0660	225.0664	-0.4	-1.8	10.5	6.6	0.0	C13 H9 N2 O2

Fig. S5 HRMS (ESI) spectrum of Compound 1.

Step 2:



2

Compound 1 (0.25 g, 1.1 mmol), D-threoninol (0.10 g, 0.96 mmol), DCC (0.22 g, 1.1 mmol) and HOBt (0.14 g, 1.1 mmol) were dissolved in anhydrous DMF (8 ml). After stirring at room temperature for 24 h, the solvent was removed under reduced pressure. The crude product was dissolved in MeOH, absorbed on silica gel, and purified by flash chromatography (gradient: ethyl acetate/methanol = 25:1) to give Compound 2 (0.24 g, 0.77 mmol, 80% yield) as a scarlet solid. ^1H NMR (400 MHz, $CDCl_3$); δ = 7.96-7.38 (m, 9H, ArH), 7.12 (d, 1H, -NHCO), 4.33 (m, 1H, -CH(OH)CH_3), 4.09 (m, 1H, $HOCH_2CH$(NHCO-)-), 3.98 (d, 2H, -CH_2-OH), 1.29 (d, 3H, -CH(OH)CH_3). ^{13}C NMR (101 MHz, CDCl3) δ 167.74, 154.34, 152.51, 135.82, 131.64, 129.18, 128.14, 123.12, 122.93, 68.87, 64.87, 55.29, 20.67. HRMS (ESI, *m/z*): calcd for $C_{17}H_{19}N_3O_3Na$ ($(M+Na)^+$) 336.1324, found mass 336.1330.

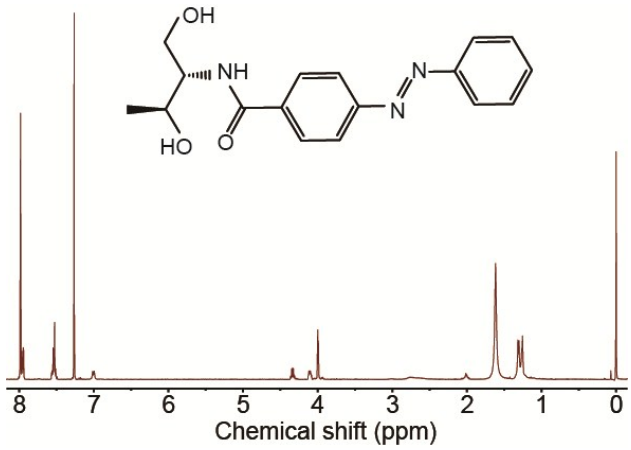


Fig. S6 ^{1}H NMR spectroscopy (400 MHz) in $CDCl_3$ of Compound 2.

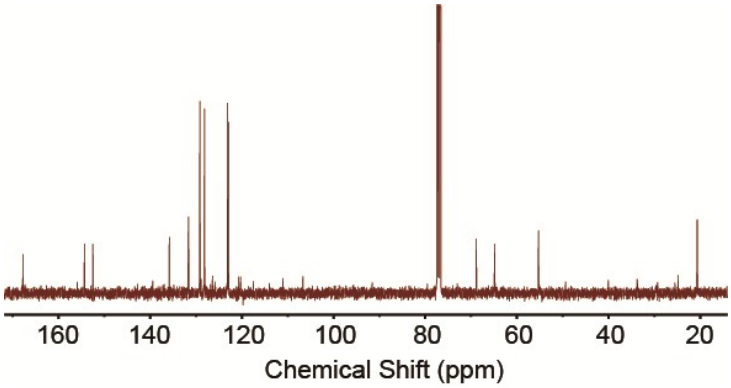


Fig. S7 ^{13}C NMR spectroscopy (101 MHz) in $CDCl_3$ of Compound 2.

Elemental Composition Report

Single Mass Analysis
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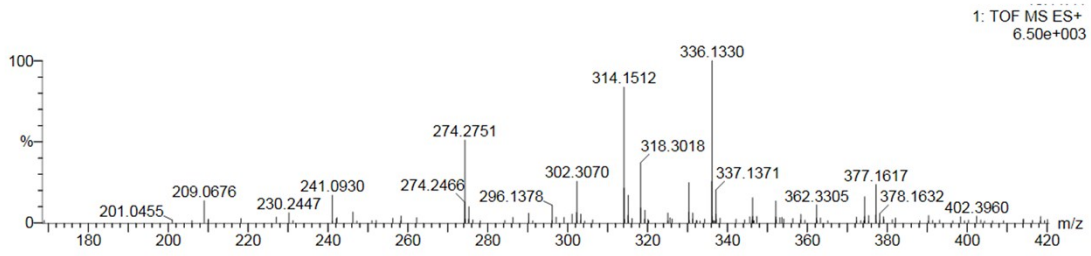
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

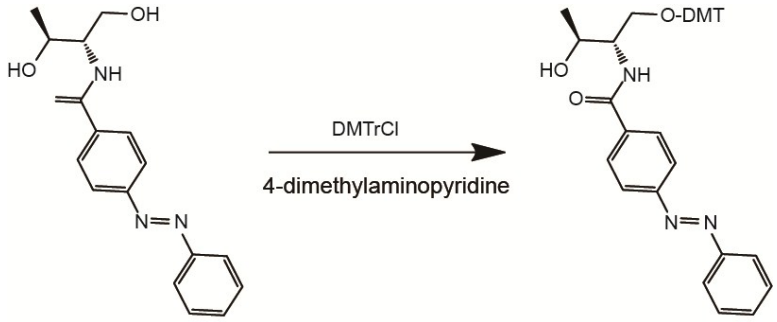
Monoisotopic Mass, Even Electron Ions
33 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-17 H: 0-42 N: 0-3 O: 0-3 Na: 0-1



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
336.1330	336.1324	0.6	1.8	9.5	66.3	0.0	C17 H19 N3 O3 Na

Fig. S8 HRMS (ESI) spectrum of Compound 2.

Step 3:



2 3

Compound 2 (0.1g, 0.31 mmol) and 4-dimethylaminopyridine (0.002 g, 0.016 mmol) were dissolve in anhydrous pyridine (5 ml) under dry Argon on an ice bath. DMTrCl (0.12 g, 0.35 mmol) in anhydrous CH_2Cl_2 (1 ml) was added to the above pyridine solution slowly under dry Argon on ice bath. The mixture was then stirring at room temperature for 24 h. The solvent was removed under reduced pressure. The crude product was dissolved in MeOH, absorbed on silica gel, and purified by flash chromatography (gradient: ethyl acetate/hexane/ trimethylamine = 50:50:1) to give Compound 3 (0.1 g, 0.19 mmol, 61% yield) as a scarlet foamy solid. ^{1}H NMR (400 MHz, $CDCl_3$); δ= 8.00-6.78 (m, 23H, ArH of DMT, azobenzene and -NHCO-), 4.25 (m, 1H, -CH(OH)CH_3), 4.17 (m, 1H, -OCH_2CH(NHCO-)-), 3.77 and 3.76 (s, 6H, -C_6H_4-OCH_3), 3.60 and 3.42 (dd, 2H, -CH_2-ODMT), 1.23 (d, 3H, -CH(OH)CH_3). ^{13}C NMR (101 MHz, CDCl3) δ 166.99, 158.68, 154.38, 152.57, 144.28, 136.08, 135.45, 135.25, 131.63, 129.93, 129.90, 129.20, 128.09, 128.02, 127.90, 127.10, 123.13, 123.01, 113.37, 86.99, 68.89, 65.49, 55.23, 53.99, 20.11. HRMS (ESI, *m/z*): calcd for $C_{38}H_{37}N_3O_5Na$ ($(M+Na)^+$) 638.2631, found mass 638.2626.

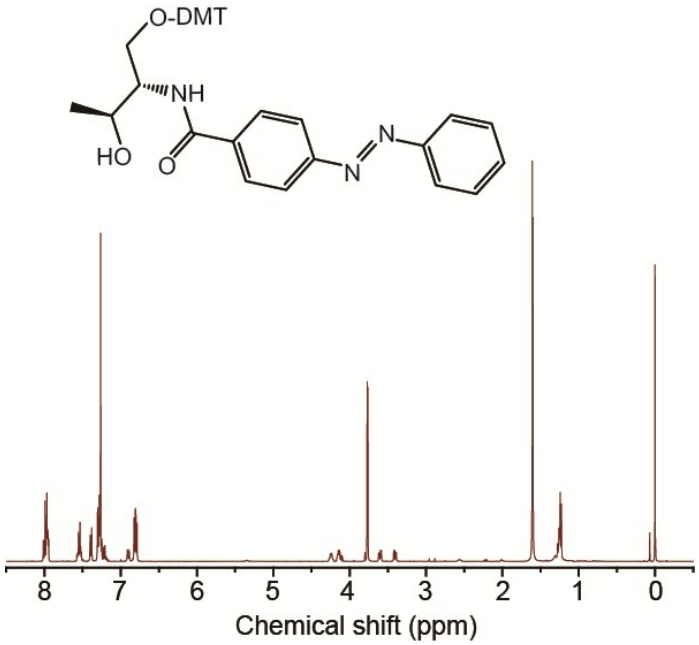


Fig. S9 ^{1}H NMR spectroscopy (400 MHz) in $CDCl_3$ of Compound 3.

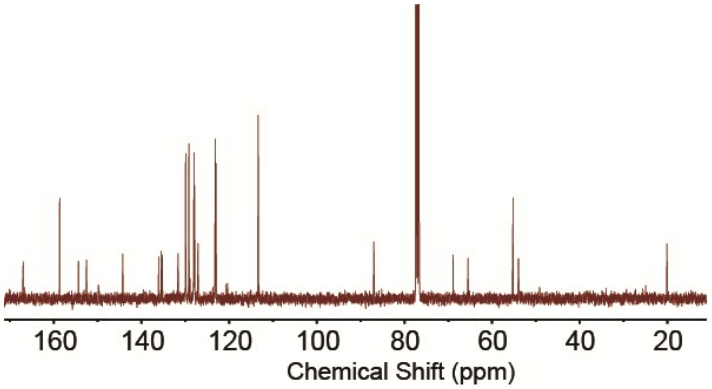


Fig. S10 ^{13}C NMR spectroscopy (101 MHz) in $CDCl_3$ of Compound 3.

Elemental Composition Report

Single Mass Analysis
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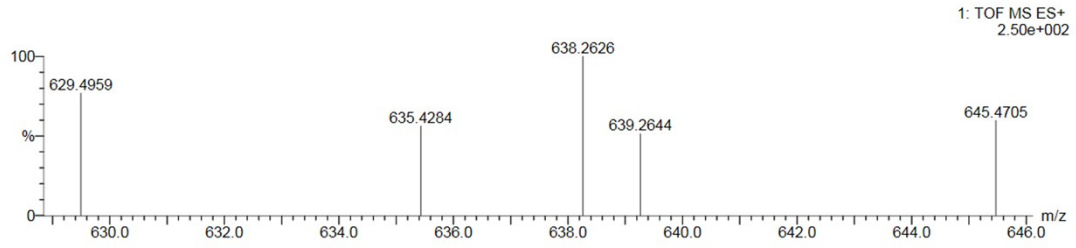
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

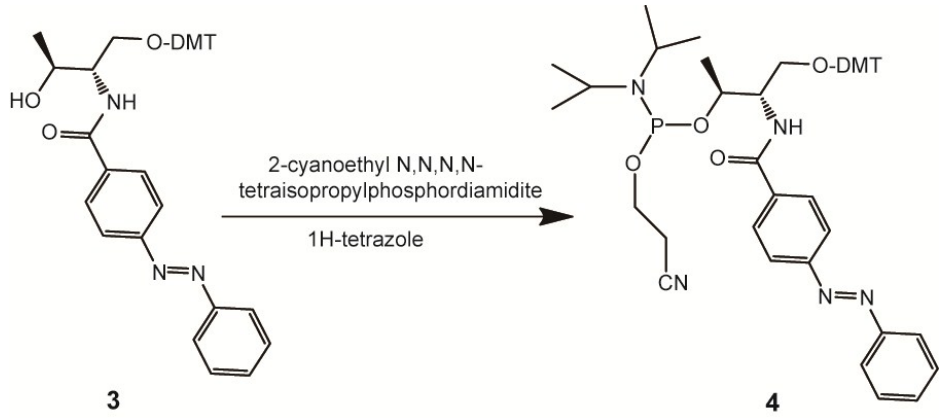
Monoisotopic Mass, Even Electron Ions
21 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-38 H: 0-200 N: 3-3 O: 0-5 Na: 0-1



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
638.2626	638.2631	-0.5	-0.8	21.5	8.0	0.0	C38 H37 N3 O5 Na

Fig. S11 HRMS (ESI) spectrum of Compound 3.

Step 4:



2-Cyanoethyl N,N,N,N-tetraisopropylphosphordiamidite (0.055 g, 0.18 mmol) was added to Compound 3 (0.1 g, 0.16 mmol) in anhydrous AN (3 ml) under dry Argon on ice bath. Then ^{1}H-tetrazole (0.014 g, 0.2 mmol) in anhydrous AN (2 ml) was added to the above AN solution slowly under dry Argon on ice bath. The mixture was

stirring at room temperature for 1 h. The solvent was removed under reduced pressure. The residual oily product was dissolve in ethyl acetate and washed by saturated aqueous solution of $NaHCO_3$ and then of NaCl. Na_2SO_4 was added to ethyl acetate solution and stirred for 30 mins. After filtration for removing Na_2SO_4, solvent was removed under reduced pressure. The crude product was dissolved in MeOH, absorbed on silica gel, and purified by flash chromatography (gradient: ethyl acetate/hexane/ trimethylamine = 40:60:1) to give Compound 4 (0.09 g,0.11 mmol, 70% yield) as a scarlet foamy solid. ^{1}H NMR ($CDCl_3$, 400 MHz); δ= 8.00-6.79 (m, 22H, ArH of DMT, azobenzene), 6.62 (d, 1H, -NHCO-), 4.48 (m, 1H, -CH(CH_3)OP-), 4.39 (m, 1H, -OCH_2CH(NHCO-)-), 3.77 and 3.76 (s, 6H, -C_6H_4-OCH_3), 3.57-3.34 (m, 4H, -CH(CH_3)$_2$, -CH_2ODMT), 2.76-2.72 (m, 2H, -CH_2CN), 1.30-1.25 (m, 15H, -CH(CH_3)$_2$, -CH(OP)CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.39, 158.38, 154.12, 152.45, 144.67, 136.34, 135.87, 135.85, 131.47, 130.05, 130.01, 129.08, 128.15, 127.88, 127.75, 126.75, 123.00, 122.83, 117.66, 113.01, 86.05, 62.63, 55.13, 54.88, 54.85, 43.16, 43.06, 24.59, 24.53, 24.35, 24.29, 20.22, 20.17, 19.72. HRMS (ESI, *m/z*): calcd for $C_{38}H_{37}N_3O_5Na$ ($(M+Na)^+$) 838.3709, found mass 838.3710.

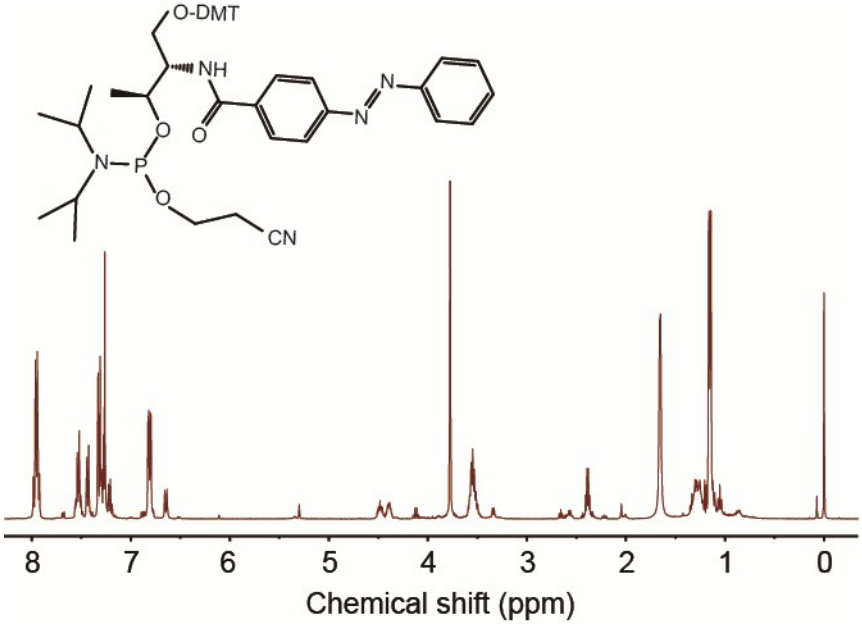


Fig. S12 ^{1}H NMR spectroscopy (400 MHz) in $CDCl_3$ of Compound 4.

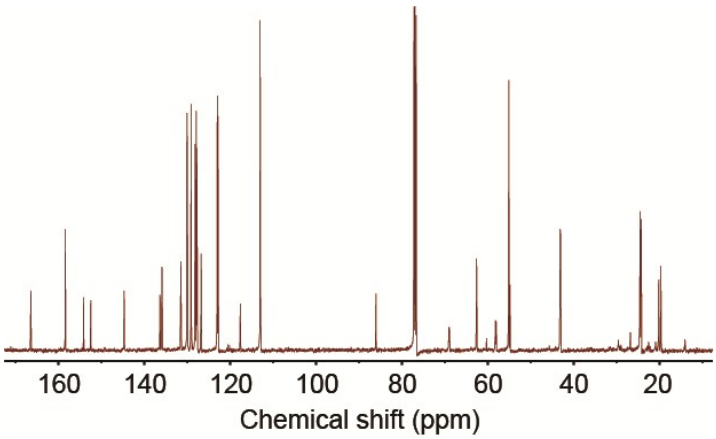


Fig. S13 ^{13}C NMR spectroscopy (126 MHz) in $CDCl_3$ of Compound 4.

Elemental Composition Report

Single Mass Analysis
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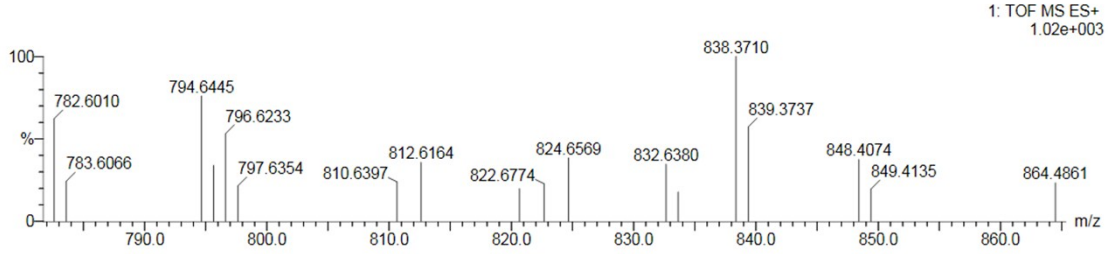
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
174 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-47 H: 0-100 N: 0-5 O: 0-6 P: 0-1 Na: 0-1



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
838.3710	838.3709	0.1	0.1	23.5	8.2	0.0	C47 H54 N5 O6 P Na

Fig. S14 HRMS (ESI) spectrum of Compound 4.

4. Nanopore analysis

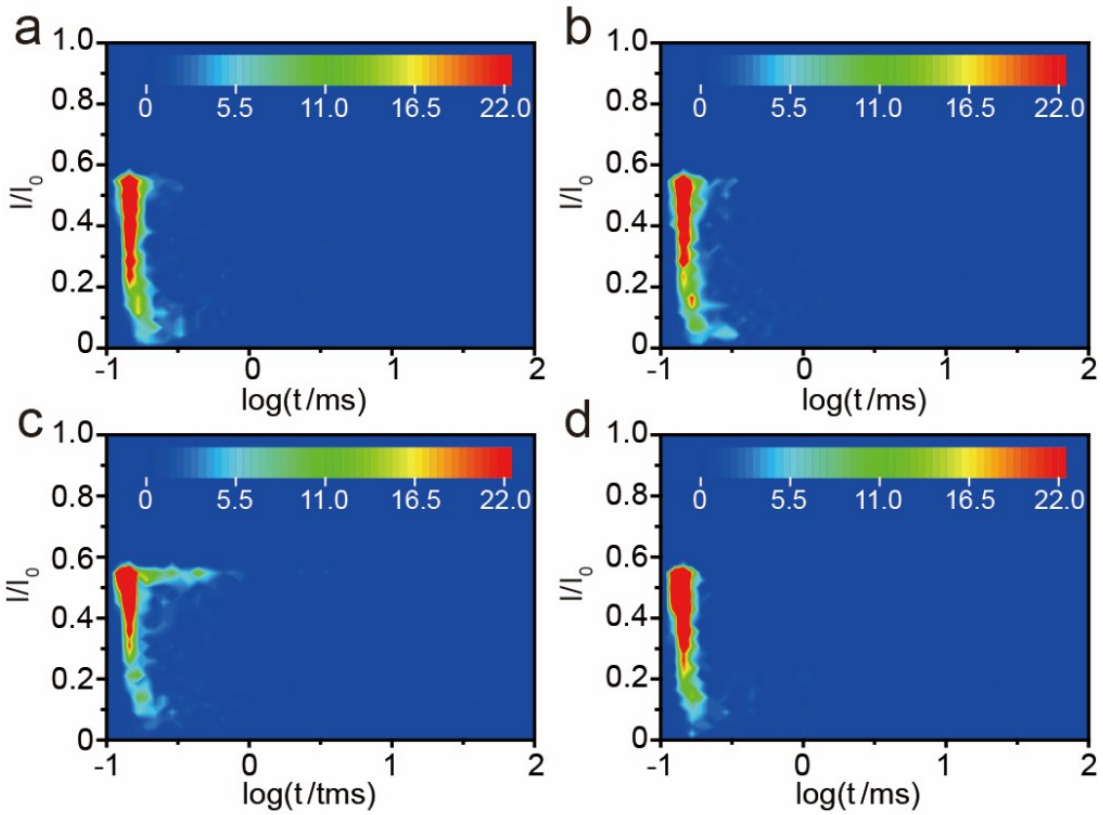


Fig. S15 The 2D contour plots for azo-ssDNA after irradiating by UV light for 5 min (a) and by *vis* light for 10 min (b), c-ssDNA (c) and single-stranded DNA (5'-CGATCCTAGC-3') for the native duplex (d). Both the plots were composed of 1350 blockage events. All the concentrations of single-stranded DNA were kept at 1 μM. The experiments were conducted with the sample added into the *cis* chamber containing 1.0 M KCl, 10 mM Tris and 1 mM EDTA (pH = 8.0) at +80 mV.

Table S1. The logarithm fitting parameters for the unzipping duration times of azo-dsDNA after UV or *vis* irradiation through the *α*-HL nanopore at different potentials.*

Voltage/mV	UV			*vis*	
	P1/ms	P2/ms	R^2	P2/ms	R^2
70	0.58 ± 0.02	12.01 ± 0.40	0.93 ± 0.02	12.59 ± 0.40	0.89 ± 0.02
80	0.52 ± 0.03	8.25 ± 0.46	0.97 ± 0.02	7.89 ± 0.26	0.95 ± 0.02
90	0.44 ± 0.02	4.57 ± 0.38	0.96 ± 0.01	4.73 ± 0.28	0.96 ± 0.02
100	0.43 ± 0.02	3.39 ± 0.29	0.96 ± 0.02	3.11 ± 0.29	0.97 ± 0.02
110	0.34 ± 0.03	2.01 ± 0.23	0.98 ± 0.02	2.00± 0.20	0.98 ± 0.02

*All the most probable unzipping times were obtained via Gaussian function and the error bars were acquired based on three separate experiments. When *vis* irradiated, the population P1 accounted for a small proportion and the fitted parameters had big deviation, thus neglected in the table. The most probable unzipping times for P2 used in the main text is the average value of the two fitted most probable unzipping times under UV and *vis* irradiation.

Table S2. The fitting frequencies and parameters based on the linear relationship between the cumulative event number and the recording time for P1 and P2 in the 2D contour plot at +80 mV. *

Irradiation	population	Frequency/ min^{-1}	R^2
UV	P1	6.0 ± 1.2	0.98 ± 0.01
	P2	6.5 ± 0.9	0.99 ± 0.01
vis	P1	2.8 ± 0.5	0.98 ± 0.01
	P2	9.6 ± 1.6	0.99 ± 0.01

*All the error bars were acquired based on three separate experiments.

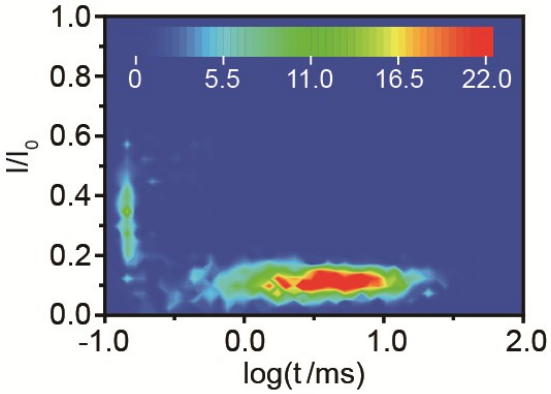


Fig. S16 The 2D contour plots of the native DNA duplex. The plot was composed of 2100 blockage events. The experiments were conducted in the solutions containing 1.0 M KCl, 10 mM Tris and 1 mM EDTA (pH = 8.0) at +80 mV.

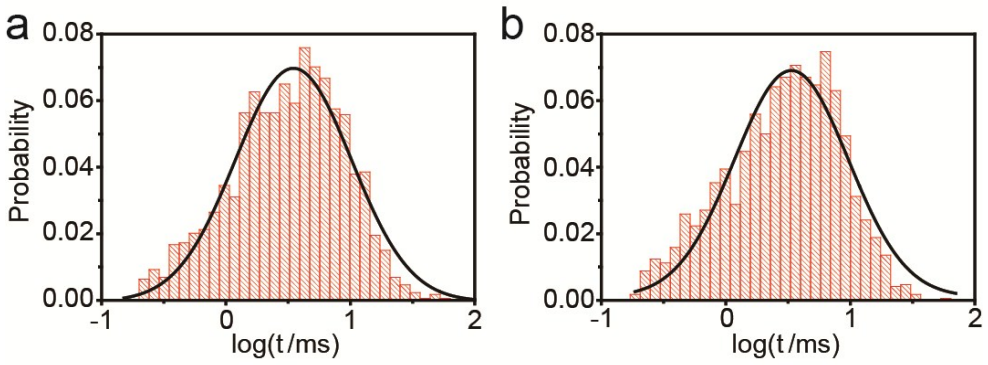


Fig. S17 Single exponential distribution of duration for the unzipping events induced by native DNA duplex after irradiated by UV for 5 min at 58 ℃ (a) and *vis* for 10 min at room temperature (b) through α-HL nanopore at +80 mV. The experiments were conducted in the solutions containing 1.0 M KCl, 10 mM Tris and 1 mM EDTA (pH = 8.0).

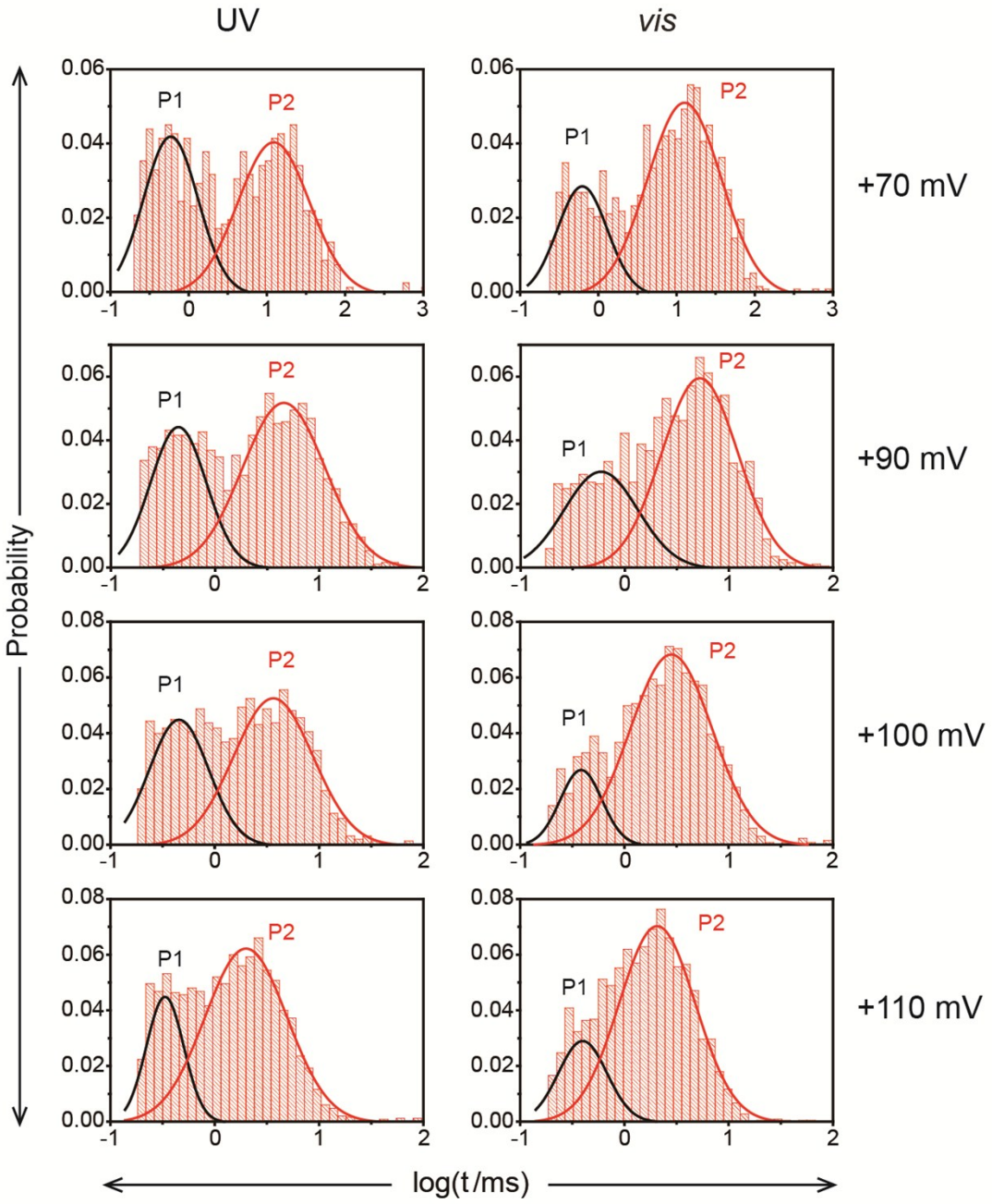


Fig. S18 Double exponential distribution of the duration for the unzipping events induced by the azobenzene-modified DNA duplex through the α-HL nanpore after irradiating by UV light for 5 min and by *vis* light for 10 min at different potentials. The experiments were conducted in the solutions containing 1.0 M KCl, 10 mM Tris and 1 mM EDTA (pH = 8.0).

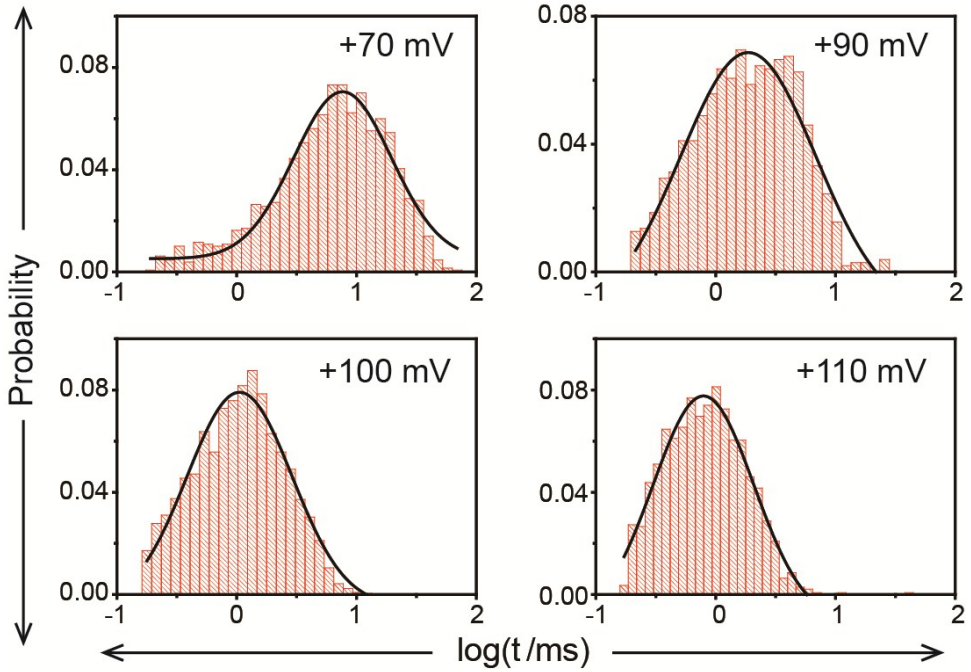


Fig. S19 Single exponential distribution of the duration for the unzipping events induced by native DNA duplex through α-HL nanopore at different potentials. The experiments were conducted in the solutions containing 1.0 M KCl, 10 mM Tris and 1 mM EDTA (pH = 8.0).

Table S3. The logarithm fitting parameters for the unzipping duration times of azo-dsDNA after UV or *vis* irradiation through the α-HL nanopore at different potentials. *

Voltage/mV	τ/ms	R^2
70	7.59 ± 0.35	0.95 ± 0.01
80	3.48 ± 0.47	0.96 ± 0.03
90	1.86 ± 0.15	0.93 ± 0.02
100	1.06 ± 0.10	0.96 ± 0.01
110	0.78 ± 0.08	0.96 ± 0.02

*All the most probable unzipping times were obtained via Gaussian function and the error bars were acquired based on three separate experiments.

Table S4. The fitting parameters for the unzipping constants τ at different potentials by single exponential function for *cis* and *trans* form of azo-dsDNA and the native duplex, respectively.

dsDNA	R^2
trans form	0.99
native	0.99
cis form	0.94

Reference

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