# DNA-Enforced Conformational Restriction of an Atropisomer

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## SUPPLEMENTARY SUPPORTING INFORMATION

## Materials and Methods

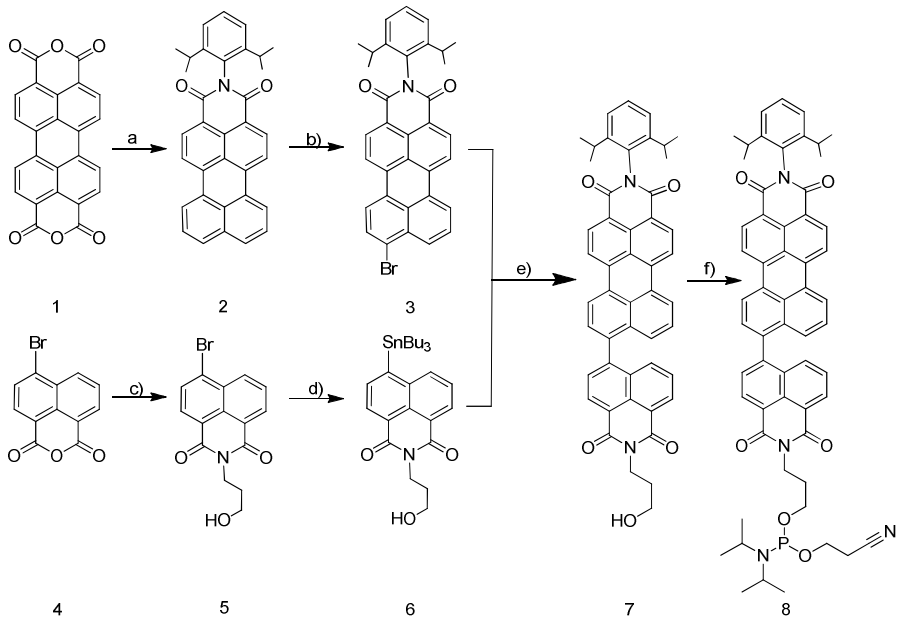
All chemicals were obtained from commercial suppliers and used as received without further purification. All reactions were carried out in glassware oven-dried prior to use and wherever necessary, were performed under dry nitrogen in dried, anhydrous solvents using standard gastight syringes, cannulae, and septa. Solvents were dried and distilled by standard procedures. TLC analyses were performed on precoated aluminum plates of silica gel 60 F254 plates (0.25 mm, Merck) and developed TLC plates were visualized under short and long wavelength UV lamps. Flash column chromatography was performed using silica gel of 200-400 mesh employing a solvent polarity correlated with the TLC mobility observed for the substance of interest. Yields refer to chromatographically and spectroscopically homogenous substances. Melting points were obtained using a capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu IRPrestige-21 FT-IR spectrometer as neat thin films between NaCl plates in case of liquids and as KBr pellets in the case of solids. $^{1}H$ and $^{13}C$ NMR spectra were measured on a 500 MHz Bruker advanced DPX spectrometer. Internal standard used for $^{1}H$ and $^{13}C$ NMR is 1,1,1,1-tetramethyl silane (TMS) and that for $^{31}P$ NMR is 85% $H_3PO_4$. All CHN analyses were carried out on an Elementar vario MICRO cube Elemental Analyzer. All values recorded in elemental analyses are given in percentages.

Oligonucleotides were synthesized on a K&A Laborgeraete DNA synthesizer. Oligonucleotides were synthesized on 1 μmol scale with appropriate controlled pore glass (CPG) beads used as 3' solid support using conventional phosphoramidite chemistry, following the procedure of Letsinger and Wu.[1] Oligonucleotides thus synthesized were isolated as trityl-on derivatives and purified using Reverse Phase High Performance Liquid Chromatography performed using a Shimadzu Prominence Liquid Chromatograph with a Phenomenex column (Luna 5u C18(2) 100A, 250 x 10 mm) with a gradient of 20 mM ammonium acetate buffer and acetonitrile with a flow rate of 1 ml/min. Molecular masses of the oligonucelotides were determined following desalting by means of MALDI-TOF mass spectroscopy on a Bruker Apex III MALDI spectrometer. UV-Visible spectrophotometry, circular dichroism, fluorescence anisotropy experiments etc. were performed using solutions of the chromophore attached oligonucleotide in 10 mM sodium phosphate buffer (pH 7.2) containing 100mM sodium chloride and an OD of 0.1 at 345 nm. Absorption and circular dichroism spectra were recorded on Shimadzu UV-3600 UV-VIS-NIR and Jasco J-815 spectrometers respectively. Steady state fluorescence data was acquired on a Horiba Jobin Yvon Fluorolog spectrofluorimeter. Fluorescence anisotropy measurements were carried out on an IBH picosecond single photon counting system. The fluorescence decay profiles were deconvoluted using IBH data station software version 2.1 and fitted, minimizing the $\chi^2$ values of the fit to $1 \pm 0.05$. Melting profiles obtained at 260 nm were fitted using Meltwin 3.5 program.[2]

Monte Carlo conformational search and Molecular Dynamics were carried out through simulations performed using the Schrödinger suite[3] of programs with the MacroModel v9.9 module, based on the AMBER force field. The initial structures for both MC and MD were constructed from B-form duplex DNA and the duplex alone was constrained during all the simulations. MD simulations were done over a timescale of 20 ns, sampling 1000 structures at 300 K. Dynamics were simulated using time steps of 1.5 fs and an equilibration time 1.0 ps.

## Synthesis Details

**Preparation of N-(2,6-diisopropylphenyl)-perylene-3,4-dicarboximide 2:** A solution of perylene-3,4,9,10-tetracarboxylic dianhydride (**1**) (3.66 g, 9.34 mmol), 2,6-diisopropylaniline (0.91 g, 5.12 mmol), zinc acetate (1.32 g, 7.19 mmol) and imidazole (18.70 g, 274.70 mmol) in 8 ml water was heated at 190 °C in a pressure tube for 23 h. The reaction mixture was extracted by chloroform and filtered through celite. The chloroform solution was washed with dilute HCl followed by water and concentrated under reduced pressure to give a brown-red residue which was then purified by column chromatography (silica gel, chloroform:petroleum ether 3:2) to produce compound **2** (1.291 g, 30%) as a red solid. mp> 300 °C; $^{1}H$ NMR (500 MHz, $CDCl_3$, δ): 8.58 (d, J = 8 Hz, 2H), 8.39 – 8.36 (m, 4H), 7.84 (d, J = 8 Hz, 2H), 7.57 (t, J = 8 Hz, 2H), 7.41 (t, J = 8 Hz, 1H), 7.27 (d, J = 8 Hz, 2H), 2.70 – 2.68 (m, 2H), 1.11(d, J = 7 Hz,12H); $^{13}C$ NMR (125 MHz, $CDCl_3$, δ): 163.99, 145.73, 137.46, 134.25, 131.94, 131.10,



a) 2,6-Diisopropylaniline/imidazole/zinc acetate/$H_2O$/190 °C; b) $Br_2$/chlorobenzene/50 °C; c) 3-aminopropanol/$H_2O$;70 °C; d) hexabutylditin/$Pd(PPh_3)_4$/toluene/reflux; e) $Pd(PPh_3)_4$/DMF/ 90 °C;
f) 2-cyanoethyl N,N-diisopropylchlorophosphoramidite/diisopropylethylamine/dry DCM/r.t.

**Scheme S1**.Shows the synthesis of conjugate **NP** and the corresponding phosphoramidite.

130.50, 129.44, 129.15, 127.93, 127.00, 126.97, 124.02, 123.78,120.97, 120.13, 29.19, 24.03; IR (KBr): 3061, 2962, 2926, 2868, 1693, 1654, 1589, 1568, 1500, 1467, 1408, 1357, 1294, 1246, 1197, 1178, 1136, 1029, 920, 889, 858, 831, 810, 754 $cm^{-1}$;Anal. Calcd. for $C_{34}H_{27}NO_2$: C, 84.80; H, 5.65; N, 2.91. Found: C, 84.89; H, 5.62; N, 2.96.

**Preparation of 9-Bromo-N-(2,6-diisopropylphenyl)-perylene-3,4-dicarboximide 3:** N-(2,6-diisopropylphenyl)-perylene-3,4-dicarboximide (**2**) (1.0 g, 2.07 mmol) was dissolved in 100 ml chlorobenzene with moderate heating. To this solution bromine (1.50 g, 9.50 mmol) was added and the reaction mixture heated at 50 °C for 4.5 h. Unreacted bromine was removed through purging with nitrogen flow. Chlorobenzene was removed under vacuum and the residue was purified by column chromatography (silica gel, chloroform:petroleum ether 2:3) to yield compound **3**(0.93 g, 80%) as a bright red solid. mp > 300 °C; $^1$H NMR (500 MHz, $CDCl_3$, δ): 8.60 – 8.57 (m, 2H), 8.41 – 8.37 (m, 2H), 8.32 (d, J = 8 Hz, 1H), 8.23(d, J = 8.5 Hz, 1H), 8.15 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 8 Hz, 1H), 7.64 (t, J = 7.5 Hz, 1H), 7.42 (t, J = 8 Hz, 1H), 7.28 (d, J = 8 Hz, 2H), 2.72 – 2.10 (m, 2H), 1.12(d, J = 7 Hz, 12H);$^{13}$C NMR (125 MHz, $CDCl_3$, δ): 163.88, 145.72, 136.86, 136.73, 132.97, 132.10, 132.03, 131.28, 130.96, 130.41, 130.05, 129.61, 129.48, 129.14, 129.06, 128.16, 126.64, 126.21, 124.45, 124.03, 123.78, 121.42, 120.72, 120.45, 29.18, 24.00; IR (KBr): 3061, 2962, 2926, 2868, 1693, 1654, 1589, 1568, 1500, 1467, 1408, 1357, 1294, 1246, 1197, 1178, 1136, 1029, 920, 889, 858, 831, 810, 754 $cm^{-1}$; Anal. Calcd for $C_{34}H_{26}BrNO_2$: C, 72.86; H, 4.68; Br, 14.26; N, 2.50. Found: C, 72.39; H, 4.97; N, 2.39.

**Preparation of 4-Bromo-N-(3-hydroxypropyl)-naphthalene-1,8-dicarboximide 5 :** To a solution of 4-bromo-1,8-naphthalic anhydride (**4**) (1.00 g, 3.60 mmol) in 100 ml water 3-aminopropanol (2.70 g, 36.00 mmol) was added. This reaction mixture was heated at 70 °C for 5 h. The reacion mixture was allowed to attain room temperature and subsequently filtered. The precipitate was then washed with water and dried. The crude product was then purified by column chromatography (silica gel, EtOAc:petroleum ether 1:1) to afford compound **5**(1.08 g, 90%) as a white solid. mp 125 °C; $^1$H NMR (500 MHz, $CDCl_3$, δ): 8.60 (d, J = 7 Hz, 1H), 8.52 (d, J = 8.5 Hz, 1H), 8.35 (d, J = 7.5 Hz, 1H), 7.99 (d, J = 8 Hz, 1H), 7.79 (t, J = 8 Hz, 1H), 4.27 (t, J = 6 Hz, 2H), 3.53 (t, J = 5.5 Hz, 2H), 2.13 (s, 1H), 1.92 - 1.90 (m, 2H); $^{13}$C NMR (125 MHz, $CDCl_3$, δ): 164.23, 164.22, 133.58, 132.35, 131.52, 131.19, 130.67, 130.64, 128.98, 128.16, 122.74, 121.85, 59.00, 37.01, 30.98; IR (KBr): 3473, 2972, 2939, 2862, 2358, 1695, 1647, 1589, 1570, 1438, 1340, 1332, 1232, 1180, 1126, 1074, 1039, 954, 846, 779, 767, 750, 734 $cm^{-1}$; Anal. Calcd for $C_{15}H_{12}BrNO_3$: C, 53.91; H, 3.62; N, 4.19. Found: C, 54.04; H, 3.69; N, 4.20.

**Preparation of 4-tributylstannyl-N-(3-hydroxypropyl)-naphthalene-1,8-dicarboximide 6:** A solution of 4-bromo-N-(3-hydroxypropyl)-naphthalene-1,8-dicarboximide (**5**) (0.50 g, 1.49 mmol), hexabutylditin (1.74 g, 2.99

mmol) and $Pd(PPh_3)_4$ (5.16 mg, 4.46 μmol) in 40 ml toluene was refluxed for 4 days. The solvent was then removed under reduced pressure and the residue was purified by column chromatography (silica gel, EtOAc:petroleum ether 1:9) to give the product **6** (0.45 g, 52.9%) as a viscous yellow liquid. $^1$H NMR (500 MHz, $CDCl_3$, δ): 8.56 (d, J = 7.3 Hz, 1H), 8.43 (d, J = 7 Hz, 1H), 8.08 (d, J = 8.25 Hz, 1H), 7.86 (d, J = 7 Hz, 1H), 7.72 – 7.69 (m, 1H), 4.28 (t, J = 6 Hz, 2H), 3.53 – 3.50 (m, 2H), 3.17 – 3.15 (m, 1H), 1.93 – 1.91 (m, 2H), 1.51 – 1.45 (m, 6H), 1.30 – 1.18 (m, 12H), 0.80 (t, J = 7.3 Hz, 9H) ; $^{13}$C NMR (125 MHz, $CDCl_3$, δ): 165.41, 164.88, 155.27, 137.51, 136.55, 136.01, 131.17, 129.88, 128.14, 126.61, 123.08, 122.14, 58.86, 36.70, 31.03, 29.07, 27.24, 13.58, 10.88; IR (KBr): 3606, 3506, 3018, 2958, 2927, 2852, 2358, 1708, 1695, 1651, 1585, 1508, 1444, 1365, 1344, 1232, 1224, 1176, 1126, 1045, 999, 950, 862, 788, 769, 756, 725 $cm^{-1}$; Anal. Calcd for $C_{27}H_{39}NO_3Sn$: C, 59.58; H, 7.22; N, 2.57. Found: C, 59.52; H, 6.80; N, 2.51.

**Preparation of 9-(4-(N-(3-hydroxypropyl)naphthalene-1,8-dicarboximide)yl)-N-(2,6-diisopropylphenyl)-perylene-3,4-dicarboximide 7:** A solution of9-Bromo-N-(2,6-diisopropylphenyl)-perylene-3,4-dicarboximide (**3**) (0.50 g, 0.89 mmol), 4-tributylstannyl-N-(3-hydroxypropyl)-naphthalene-1,8-dicarboximide (**6**) (0.61 g, 1.12 mmol) and $Pd(PPh_3)_4$ (10.28 mg, 8.89 μmol) in 50 ml DMFwas heated at 90 °C for 2 days. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, EtOAc:petroleum ether 1:1) to afford compound **7**(0.33 g, 50%) as a yellow-orange solid. mp> 300 °C; $^1$H NMR (500 MHz, $CDCl_3$, δ): 8.72 (d, J = 7.35 Hz, 1H), 8.67 – 8.60 (m, 3H), 8.56 (d, J = 7.9 Hz, 1H), 8.51 (d, J = 8.25 Hz, 1H), 8.46 (d, J = 8.1 Hz, 2H), 7.86 – 7.84 (m, 1H), 7.80 (d, J = 7.5 Hz, 1H), 7.62 – 7.57 (m, 2H), 7.47 – 7.40 (m, 2H), 7.35 (d, J = 8.5 Hz, 1H), 7.28 (d, J = 8 Hz, 2H), 4.37 (t, J = 6.1 Hz, 2H), 3.60 – 3.58 (m, 2H), 3.04 (s, 1H), 2.75 – 2.68 (m, 2H), 2.01 – 1.96 (m, 2H), 1.13 – 1.11 (m, 12H); $^{13}$C NMR (125 MHz, $CHCl_3$, δ): 164.82, 164.67, 163.94, 145.75, 145.72, 144.72, 139.01, 137.27, 133.41, 132.91, 132.17, 132.14, 131.91, 131.15, 130.95, 130.10, 129.75, 129.53, 129.16, 129.06, 128.86, 128.56, 128.29, 127.70, 127.41, 126.99, 124.14, 124.07, 123.15, 122.71, 122.41, 121.41, 120.80, 120.68, 59.00, 36.98, 31.07, 29.19, 24.05, 24.03; IR (KBr): 3466, 2960, 2927, 2866, 2358, 1699, 1658, 1591, 1577, 1467, 1444, 1359, 1244, 1178, 1132, 1056, 1039, 844, 812, 786, 758, 748 $cm^{-1}$; Anal. Calcd for $C_{49}H_{38}N_2O_5$: C, 80.09; H, 5.21; N, 3.81. Found: C, 80.59; H, 5.21; N, 3.85.

**Preparation of O-(2-Cyanoethyl)-N,N-diisopropyl-O-[9-(4-(N-(3-hydroxypropyl)naphthalene-1,8-dicarboximide)yl)-N-(2,6-diisopropylphenyl)-perylene-3,4-dicarboximide]phosphoramidite8:** A solution of 9-(4-(N-(3-hydroxypropyl)naphthalene-1,8-dicarboximide)yl)-N-(2,6-diisopropylphenyl)-perylene-3,4-dicarboximide (**7**) (0.50 g, 0.68 mmol), diisopropylethylamine (0.307 g, 2.38 mmol) and, 2-cyanoethyl N, N -diisopropylchlorophosphoramidite (0.241 g, 1.02 mmol) in 25 ml dry DCM was stirred for an hour at room temperature. The reaction mixture was then quenched by dry methanol. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, DCM:triethylamine 9:1) to afford compound **8** (0.635 g, 90%) as a red-orange solid. mp> 300 °C; $^1$H NMR (500 MHz, $CD_2Cl_2$, δ): 8.66 (d, J = 7.4 Hz, 1H), 8.63 – 8.59 (m, 3H), 8.56 – 8.54 (m, 2H), 8.51 – 8.48 (m, 2H), 7.81 – 7.78 (m, 2H), 7.65 (d, J = 7.7 Hz, 1H), 7.57 – 7.54 (m, 1H), 7.47 – 7.41 (m, 2H), 7.36 (d, J = 8.35 Hz, 1H), 7.28 (d, J = 7.8 Hz, 2H), 4.29 – 4.24 (m, 2H), 3.81 – 3.74 (m, 4H), 3.55 – 3.51 (m, 2H), 2.71 – 2.67 (m, 2H), 2.59 – 2.57 (m, 2H), 2.04 – 2.00 (m, 2H), 1.12 – 1.11 (m, 2H), 1.08 – 1.06 (m, 12H); $^{13}$C NMR (125 MHz, $CD_2Cl_2$, δ): 164.42, 164.39, 146.53, 144.57, 139.80, 137.82, 137.57, 133.82, 132.81, 132.34, 131.92, 131.56, 130.97, 130.89, 130.23, 129.95, 129.72, 129.63, 129.44, 129.38, 128.90, 128.63, 127.95, 127.59, 127.35, 124.65, 124.42, 123.79, 123.56, 123.29, 121.78, 121.67, 121.21, 121.11, 118.36, 62.04, 58.98, 58.83, 43.52, 43.42, 38.38, 29.49, 24.11, 20.83, 20.78; $^{31}$P NMR(250 MHz, $CD_2Cl_2$, δ): 147.64.

## References


[1] R. L. Letsinger, T. Wu, *J. Am. Chem. Soc.***2005**, *127*, 4172.4173.
[2] J. A. McDowell, *Meltwin 3.5*; 2001.
[3] Schrodinger-Inc. Portland, 2011-2012.

**Table S1**. m/z values for hairpin sequences **ODN1** and **ODN2** determined by MALDI-TOF mass spectrometry.

| | m/z Calculated | m/z Found |
|---|---|---|
| **ODN1** | 5649.4 | 5654.4 |
| **ODN2** | 4854.2 | 4856.6 |

**Table S2**. Thermodynamic data[a] ofthe dyad **NP** end-capped oligonucleotide **ODN1** and the model DNA hairpin **ODN2**.

| | $T_m$ ($^oC$) | $\Delta T_m$ ($^oC$)[b] |
|---|---|---|
| **ODN2** | 38.80 | - |
| **ODN1** | 40.14 | 1.34 |

[a]As analysed using Meltwin from absorbance at 260 nm during thermal dissociation in 10mM phosphate buffer (pH 7.2) containing 100mM NaCl. [b]The difference in melting points of **NP** attached oligonucleotide **ODN1**and the model hairpin DNA **ODN2**.

**Table S3**. Photophysical properties of model chromophore **NP**[a] in DMSO and **NP** end-capped DNA **ODN1** in 10 mM phosphate buffer (pH 7.2) containing 100 mM NaCl.

| | $\lambda_{abs}$ nm ($\varepsilon$, $M^{-1}$ $cm^{-1}$) | $\lambda_{ems}$, nm | $\Phi$ | $\tau$, ns (Amplitude in %) |
|---|---|---|---|---|
| **NP** | 340.0 (1.63 x $10^4$)<br>515.0 (4.60 x $10^4$) | 575 | 0.7 | 3.79 (100) |
| **ODN1** | 260.0 (2.39 x $10^5$)<br>349.0 (2.16 x $10^4$)<br>525.5 (6.65 x $10^4$) | 602 | 0.12 | 3.29 (70)<br>1.08 (30) |

[a]Photophysical data measured in dimethylsulfoxide due to solubility reasons in buffer. $\Phi$ isfluorescence quantum yield and $\tau$ is fluorescence lifetime.

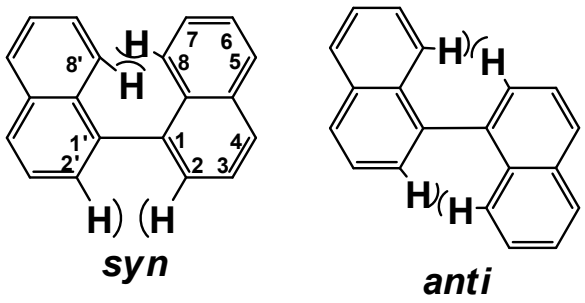



**Figure S1**. Shows two possible pathways for racemisation of 1,1'-binaphthalene.

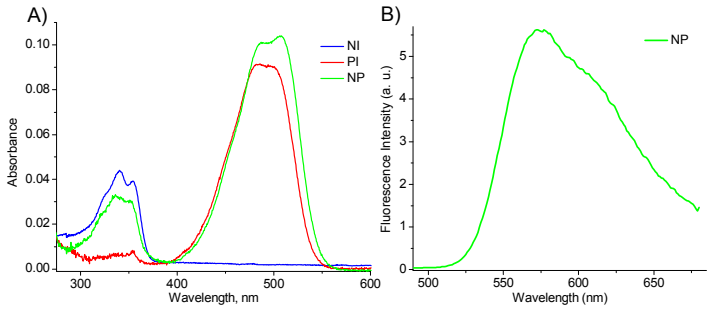



**Figure S2**. Shows the A) absorption spectra of the naphthalenimide – perylenimide dyad **NP** and the model naphthalenimide **NI** and perylenimide **PI** derivatives and B) fluorescence spectrum of naphthalenimide-perylenimide dyad **NP**in DMSO.

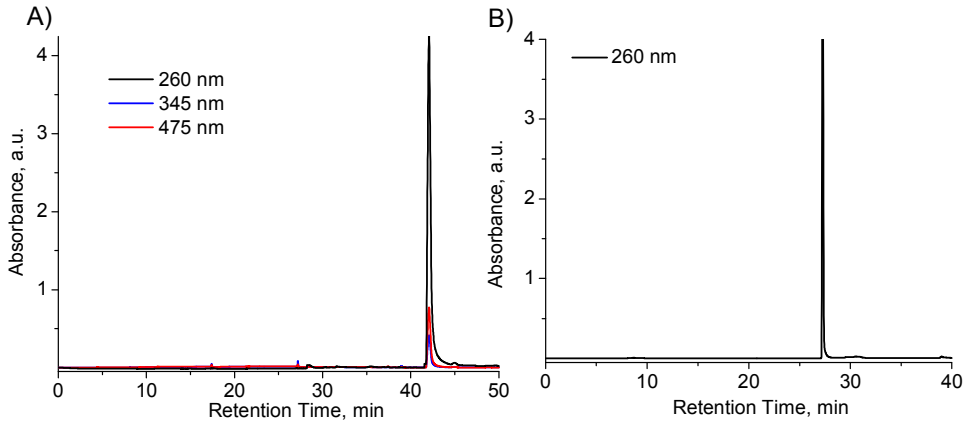



**Figure S3**.RP-HPLC trace of A) **NP** end-capped DNA hairpin **ODN1** and B) model DNA hairpin **ODN2**.

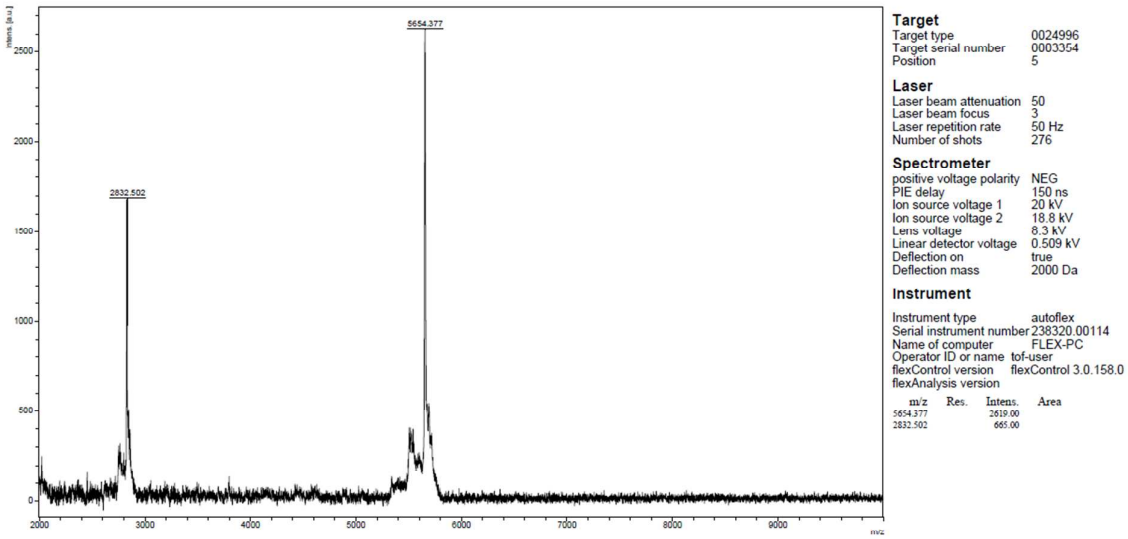



**Figure S4**.MALDI-TOF mass spectrum of representative **NP** end-capped DNA hairpin **ODN1**.

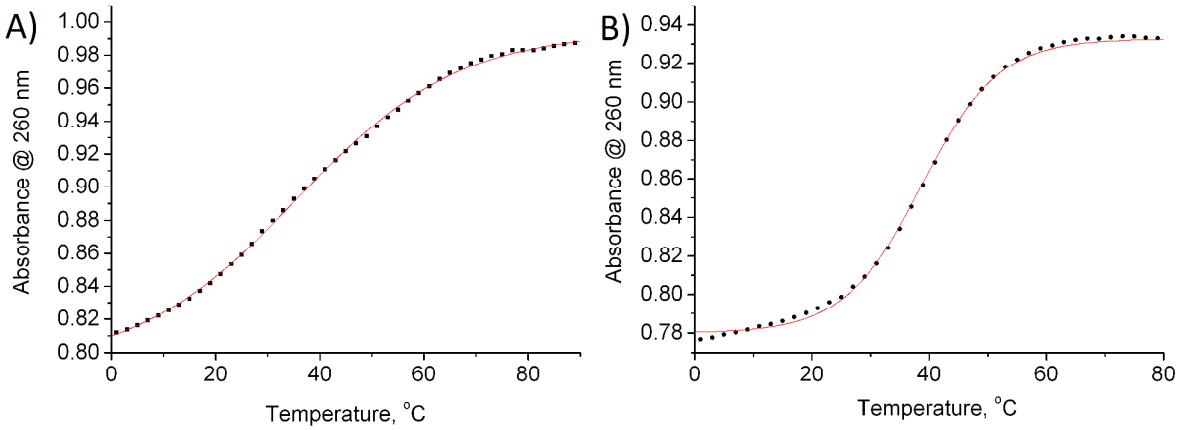



**Figure S5**.Thermal denaturation curve of A) **NP** end-capped DNA hairpin **ODN1** and B) the model DNA hairpin **ODN2**.

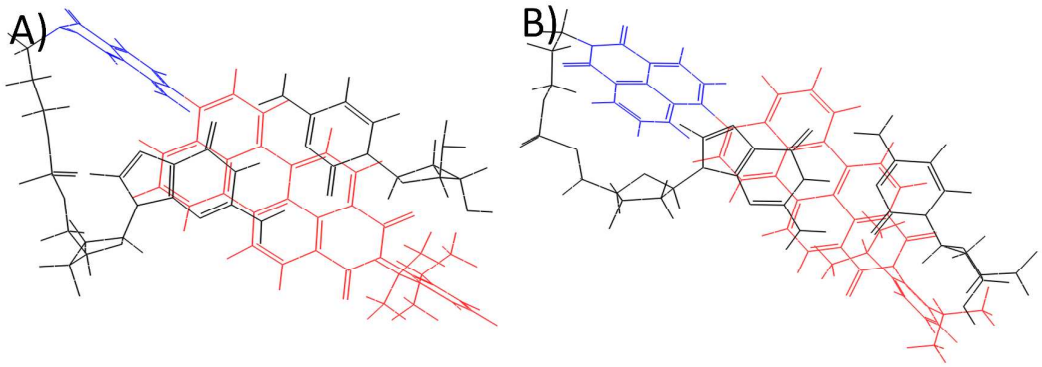



**Figure S6.** Shows the two stacking interactions between the adjacent base pair of the hairpin (GC) and the dyad **NP** in A) the (P) atropisomer and B) the (M) atropisomer.

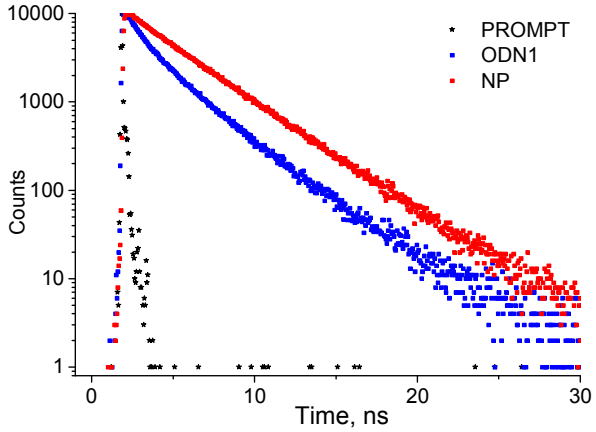



**Figure S7**.Time-resolved fluorescence spectra of **NP** in DMSO (Red) and **NP** end-capped hairpin DNA **ODN1** (Blue) in 10 mM phosphate buffer (pH 7.2) containing 100 mM NaCl recorded at 0 °C. Excitation wavelength is 439 nm and emission is monitored at 602 nm.

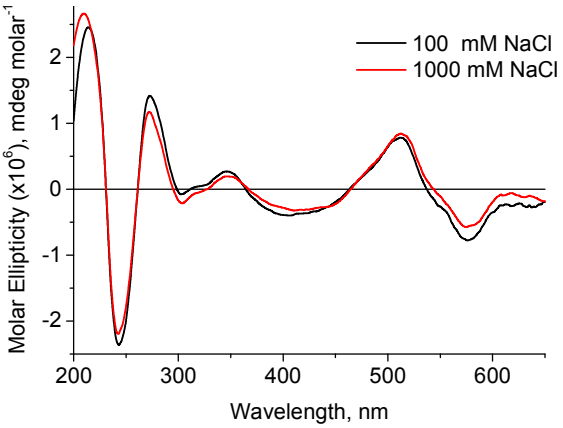



**Figure S8.** Shows the CD spectra of **ODN1** in 10 mM phosphate buffer (pH 7.2 ) containing 100 mM (black) and 1000 mM (red) NaCl.