Supplementary Information

Programming DNA and PNA quadruplex formation by π-π-stacking interactions

Sourav Saha,[a] Jianfeng Cai,[a] Daniel Eiler,[a] and Andrew D. Hamilton*[a]

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[a]*Department of Chemistry, Yale University, 225 Prospect Street, New Haven, CT 06520, USA*
Email: andrew.hamilton@yale.edu

Circular Dichroism of Unmodified $G_4T_4G_4$ DNA, PNA, and Their Hybrid Quadruplexes.
The CD spectrum of the unmodified $G_4T_4G_4$ DNA showed a peak at 295 nm and a trough at 265 nm, characteristic of an antiparallel DNA quadruplex (Fig. S1). The same for a homologous PNA quadruplex did not show any CD signal because the PNA-backbone lacks a chiral center. A 1:1 mixture of homo DNA and PNA quadruplexes did not result in any change in their composition and conformation, as evidenced from an unchanged CD profile, except from a 50% reduction in intensity as a result of a two-fold dilution. In contrast, annealing of the 1:1 mixture of the DNA and PNA quadruplexes in which each strand had a concentration of 10 μM, the characteristic signals for antipapallel DNA quadruplex disappeared and a new peak at 265 nm and a trough at 245 nm emerged, indicating the formation of a hybrid DNA_2-PNA_2 parallel quadruplex (Also see: Armitage et al. *J. Am. Chem. Soc*. 2003, **125**, 4111).

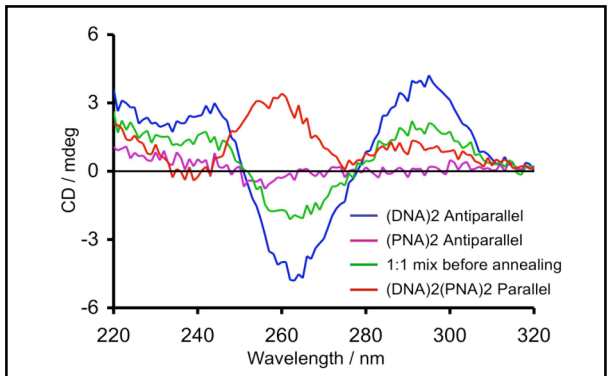


Fig. S1 CD spectra demonstrating the formation of unmodified $G_4T_4G_4$ DNA, PNA, and their hybrid quadruplexes formed at 5 μM quadruplex concentration.

CD Melting Results of 3-DNA and 4-DNA Quadruplexes.
To determine the thermal stability of the **3**-DNA and **4**-DNA quadruplexes formed from 20 and 40 μM single-strand (SS) concentrations, the intensity of the 265 nm CD peak was monitored against increasing temperature between 25˚C and 95˚C (Fig. S2). The parallel quadruplex formed by **4**-DNA at both concentrations dissociated gradually, which was complete at 95 ˚C, as the intensity of the 265 nm CD peak reached zero. On the other hand, the 265 nm CD signal for the **3**-DNA quadruplex formed at each concentration disappeared partially even at 95 ˚C as a result of a partial dissociation of the corresponding tetra-

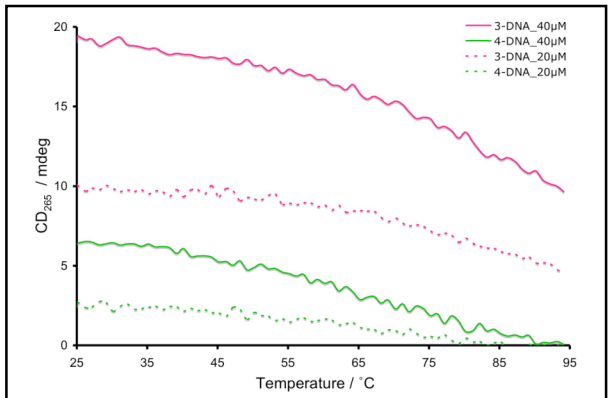
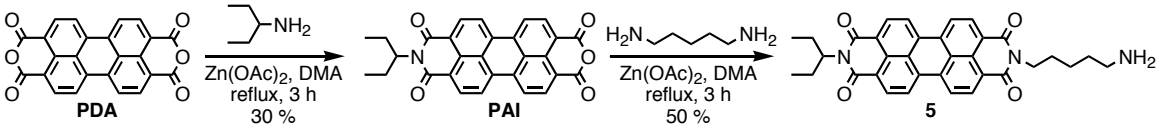


Fig. S2 Thermal dissociation of **3**-DNA and **4**-DNA quadruplexes showing partial dissociation of the former and complete dissociation of the latter at 95 ˚C.

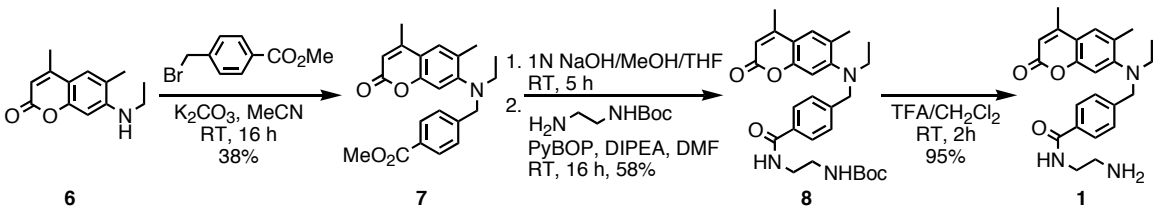
molecular assembly. This again suggests that eight G-quartet planes in the parallel tetramolecular **3**-DNA quadruplex stabilise the corresponding ODN assembly more strongly than four G-quartet planes in the dimeric assembly of **4**-DNA.

General Methods. Starting materials and reagents were purchased from Aldrich and used as received. Syntheses of **1** and **5** starting from coumarin-2 (**6**) and perylene-3,4,9,10-dicaboxyanhydride (PDA), respectively, are reported here. Synthesis of **3** is reported elsewhere.[1] Resin bound 5′-modified $G_4T_4G_4$ DNA oligomer and FmocNH-$[G(bhoc)]_4T_4[G(bhoc)]_4$-LysNHBoc PNA were purchased from Yale Keck Foundations and Biosyn Inc., respectively. Analytical thin-layer chromatography (TLC) was performed on aluminium sheets, precoated with silica gel 60-F_{254} (Merck 5554). Flash chromatography was performed with silica gel 60 (Silicycle). ^{1}H and ^{13}C NMR Spectra were recorded on Bruker Avance 500 MHz spectrometer and Bruker 125 MHz spectrometer, respectively, at ambient temperature in appropriate deuterated solvents using tetramethylsilane as an internal reference. Matrix-assisted-laser-desorption-ionization mass spectra (MALDI-MS) were recorded on an Applied Biosystems Voyager System 6268 mass spectrometer. Circular dichroism spectra were recorded by Aviv Instruments Circular Dichroism Spectrometer 202 and fluorescence spectra were recorded by Hitachi F-4500 Fluorescence Spectrophotometer.

Synthesis of 5. Perylene diimide (PDI) derivative **5** was prepared by following literature procedures,[2] starting from **PDA** which was first converted to the corresponding mono-imide (**PAI**) with 1-ethylpropylamine. A degassed solution of **PAI** (0.46 g, 1 mmol), 1,5-diaminopentane (1.02 g, 10 mmol), and $Zn(OAc)_2$ (0.364 g, 2 mmol) in 10 mL of *N,N*-dimethylacetamide was heated at 50˚C for 3 h under N_2. Solvent was evaporated under reduced pressure. Pure PDI derivative **5** was obtained after column chromatography (SiO_2, CH_2Cl_2 to 95:5 CH_2Cl_2/MeOH) as a red solid (0.27 g, 50%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.58 (d, 4H), 8.50 (d, 4H), 4.95 (m, 1H), 4.15 (t, 2H), 2.67 (t, 2H), 2.20 (m, 2H), 1.87 (m, 2H), 1.72 (m, 2H), 1.60–1.40 (m, 4H), 0.85 (t, 6H) ppm. MALDI-TOF MS (*m/z*): calculated ($C_{34}H_{32}N_3O_4$) $[M+H]^+$: 546.24, found 546.45.



Synthesis of 7. Compound **6** (0.5 g, 2.3 mmol), Methyl 4-bromomethyl benzoate (0.412 g, 1.8 mmol), and K_2CO_3 (0.317 g, 2.3 mmol) were placed in 100 mL round bottom flask, and then to which 20 mL anhydrous MeCN was added. The mixture was refluxed overnight, and then dissolved in 100 mL EtOAc and washed with NH_4Cl (1×20 mL), H_2O (2×20 mL) and brine 20 mL. The organic layer was dried and evaporated, and purified by flash chromatography (SiO_2, 1:2 EtOAc/Hexane) to obtain **2** (0.25 g, 38%) as a white foam solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.95 (d, J = 8.19 Hz, 2H), 7.38 (s, 1H), 7.36 (d, J = 8.19 Hz, 2H), 6.91 (s, 1H), 6.13 (s, 1H), 4.26 (s, 2H), 3.89 (s, 3H), 3.08 (q, J = 6.94 Hz, 2H), 2.41 (s, 3H), 2.37 (s, 3H), 1.08 (t, J = 6.94 Hz, 3H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.9, 161.44, 153.4, 152.6, 152.3, 143.7, 129.7, 129.5, 129.1, 128.0, 126.6, 115.0, 112.8, 109.3, 56.4, 52.0, 46.9, 18.6, 11.9 ppm. HR-ESI MS (*m/z*): calculated ($C_{22}H_{23}NO_4$) $[M + H]^+$ 366.1700, found 366.1703.



Synthesis of 8. Compound **7** (0.25 g, 0.68 mmol) was dissolved in 30 mL 1N NaOH in 2:1:2 H_2O/MeOH/THF and stirred for 5 h. To the reaction mixture, 100 mL CH_2Cl_2 was added and washed with 100 mL H_2O. The organic layer was separated and dried over anhydrous Na_2SO_4, and evaporated to give a yellowish solid. To this product was added Boc-aminoethyl amine (0.119 g, 0.75 mmol), PyBop (0.39 g, 0.75 mmol), and 20 mL DMF, followed by 0.44 mL, 2.5 mmol DIPEA. The mixture was stirred overnight. To the reaction mixture 200 mL CH_2Cl_2 was added and washed with 200 mL H_2O. The organic layer was separated, dried, and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, 4:3 EtOAc/Hexane) to provide **8** (0.2 g, 58% in two steps) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.75 (d, J = 7.88 Hz, 2H), 7.36 (s, 1H), 7.33 (d, J = 8.19 Hz, 2H), 6.89 (s, 1H), 6.12 (s, 1H), 4.23 (s, 2H), 3.53 (m, 2H), 3.37 (m, 2H), 3.06 (q, J = 6.94 Hz, 2H), 2.40 (s, 3H), 2.37 (s, 3H), 1.40 (s, 9H), 1.08 (t, J = 6.94 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.6, 161.5, 153.5, 152.6, 142.0, 133.1, 129.6, 128.1, 127.2, 126.6, 115.0, 112.7, 109.3, 79.8, 56.3, 46.9, 41.8, 40.1, 28.3, 18.6, 11.8. HR-ESI MS (*m/z*): calculated ($C_{29}H_{25}N_3O_5$) $[M + H]^+$ 494.2649, found 494.2649.

Synthesis of 1. Compound **8** (0.2 g, 0.4 mmol) was dissolved in 30 mL 1:1 TFA/CH_2Cl_2 and stirred for 2 h. The solvent was removed and the residue was lyophilized to provide **1** (0.15 g, 95%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.51 (s, 1H), 7.76 (m, 5H), 7.53 (s, 1H), 7.42 (d, J = 8.19 Hz, 2H), 6.98 (s, 1H), 6.18 (s, 1H), 4.36 (s, 2H), 3.48 (m, 2H), 3.10 (m, 2H), 2.97 (q, J = 6.94 Hz, 2H), 2.38 (s, 3H), 2.37 (s, 3H), 1.07 (t, J = 6.94 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 167.1, 160.6, 158.8, 158.6, 158.5, 153.5, 152.3, 142.5, 133.1, 129.4, 128.2, 127.7, 127.4, 117.2, 114.6, 112.1, 109.0, 54.8, 47.6, 37.4, 18.6, 12.2. HR-ESI MS (*m/z*): calculated ($C_{23}H_{27}N_3O_3$) $[M + H]^+$ 394.2125, found 394.2126.

5′-End functionalization of $G_4T_4G_4$ oligonucleotides (ODNs)

To the compounds **1**, **3**, or **5** (10 mg, each), placed in separate vials, 1 mL of anhydrous DMF and 50 μL DIPEA were added. The solution was transferred into syringes attached to cartridges containing resin-bound activated C10-N-hydroxysuccnimide (NHS) ester modified $G_4T_4G_4$ ODNs (1.0 μmol) for solid-phase peptide coupling reactions. The solution was pushed through the cartridge every 15 min for three times, and then agitated overnight. After removing reagent solutions from cartridges, functionalized resin-bound ODNs were washed with DMF (10×3 mL) and MeCN (10×3 mL). The resin was dried by blowing Ar through the cartridge for 1 h. 5′-Functionalized ODNs were cleaved from resin support and subjected to global deprotection using 30% NH_4OH solution (3 mL) at 55˚C for 16 h. The resulting ODNs were purified by reverse-phase HPLC (H_2O/MeCN) to obtain **1**-DNA, **3**-DNA, and **5**-DNA.

For attachment of porphyrin **4** to ODNs, resin-bound C6-amino modified $G_4T_4G_4$ ODNs (1 μmol) treated with a solution of **4** (8 mg, 10 μmol), PyBOP (21 mg, 40 μmol), and 50 μL DIPEA in 1 mL *N*-methyl-2-pyrrolidinone (NMP) in a cartridge at room temperature for 16 h, as described above. After removing the reagent solution, the solid-phase was washed with NMP (10×3 mL) and MeCN (10×3 mL). The resin was dried by blowing argon through the cartridge for 1 h. 5′-Functionalized ODNs were cleaved from resin support using 30% NH_4OH solution (3 mL) at 55˚C for 16 h. The resulting ODNs were purified by reverse-phase HPLC (H_2O/MeCN) to obtain **4**-DNA.

All 5′-functionalized ODNs were characterized by MALDI-TOF mass spectrometry (**1**-DNA: *m/z* calculated 4414, found 4414; **3**-DNA: *m/z* calculated 4584, found 4581; **4**-DNA: *m/z* calculated 4740.3, found 4747.1; **5**-DNA: *m/z* calculated 4568.1, found 4569.0)

_N_-End functionalization of $G_4T_4G_4$-K PNA

Resin-bound FmocNH-[G(bhoc)]$_4$T$_4$[G(bhoc)]$_4$-LysNHBoc PNA (100 mg) was placed in a cartridge and was treated with 1:4 piperidine/DMF (2×2 mL) to remove the Fmoc protecting group. The solid residue was washed thoroughly with DMF (10×3 mL) and MeCN (10×3 mL) and dried by blowing Ar through the cartridge for 1 h. *N*-terminal of the resin-bound PNA was subjected to peptide coupling with a solution mixture of **2** (0.1 M), HATU (0.2 M) and DIPEA (0.8 M) in 1 mL NMP at room temperature for 16 h. After removing the reagents by washing with NMP (10×3 mL) followed by MeCN (10×3 mL), the solid phase was dried again under Ar flow. *N*-Functionalized PNA was cleaved from resin support and Bhoc and Boc groups were removed by treatment with a 1:4 *m*-cresol/TFA solution (3×1 mL) at room temperature for 2 h. After combining *m*-cresol/TFA solutions, PNA was suspended from the solution by adding Et_2O (30 mL). The suspension was cooled in dry ice (10 min) and centrifuged for 5 min. Decantation of solvent mixture from the top provided a greenish gel at the bottom of the centrifuge-tube. It was washed thoroughly with Et_2O (4×20 mL) by repeating suspension, centrifugation, and decantation for four times. After evaporating Et_2O completely a green powder was obtained which was dissolved in H_2O and purified by reverse-phase HPLC (H_2O/MeCN) to obtain **2**-PNA. It was characterized by MALDI-TOF mass spectrometry (**2**-PNA: *m/z* calculated 3809.4, found 3810.6).

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