Allosteric control of self-assembly: modulating guanine quadruplex formation through orthogonal aromatic interactions

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Ia. General Experimental

Ib. Synthesis of 5-[4-N(3-aminopropyl amidyl) phenyl]-15- Porphyrin (P)

Ic. Standard preparation of porphyrin conjugated ODNs

Id. Experimental protocols for self-assembly of quadruplex DNA

IIa. CD spectra of 2P and 2Ph after self-assembly

IIb. CD spectra of preformed quadruplex [1P]4 before and after addition of HP-β-CD

IIc. CD and UV-vis spectra of preformed quadruplex [4P]4 before and after addition of HP-β-CD

IId. Temperature induced absolute change in ellipticity for quadruplexes [1P]4, [4P]4, non-aggregated ODN 1, and ODN 1P incubated the presence of HP-β-CD

IIe. CD spectra of ODNs 4P and 1P with HP-β-CD present during quadruplex self-assembly

IIf. CD spectra of ODN 1 self-assembled under varying incubation time-frames

III. MALDI-TOF data for ODNs 1P, 2P, 4P, SSP, 2Ph and 1Ph
Ia. General Experimental

Dichloromethane was distilled from calcium hydride and triethylamine was distilled over NaOH pellets. Reactions requiring anhydrous conditions were done in oven-dried (110 °C) glassware under atmosphere of dry N₂. Both ¹H and ¹³C NMR spectra were acquired on Bruker Advance DRX 400 or DRX 500 series spectrophotometer at 400 and 500 MHz, respectively. Preparative HPLC was carried out on a Waters 600E controller in conjunction with Waters 490E multiwavelength UV detector. Analytical HPLC was performed on a Rainin HP controller with a Rainin UV detector, both attached to a Dell Optiplex PC running Varian Star Workstation software. High Resolution Electrospray Ionization Mass spectrometry data were obtained by the University of Illinois at Urbana-Champaign, School of Chemical Science, Mass Spectrometry Laboratory.

The core oligonucleotide sequences used in this study were synthesized by the W. M. Keck Foundation Biotechnology Resource Laboratory located at Yale University, using standard automated solid phase synthesis. Modified phosphoramidite 5’-Carboxy-Modifier C10 was purchased from Glen Research. All DNA oligonucleotides were purified by gel filtration using Microspin G-25 Columns (Amersham Biosciences) followed by chromatographic separation by reverse-phase HPLC on a Varian Prostar equipped with a Timberline TL-105 column heater. The concentrations of purified oligonucleotides were quantified based on their respective electronic absorption at 260 nm and their molar extinction coefficients obtained by nearest neighbor calculations. Purified oligonucleotides
were characterized by Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) spectrometry (Applied Biosystems Voyager –DE PRO Workstation). (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD) was purchased from Sigma as a 45% (w/v) solution in water.

Formation of quadruplexes was verified by circular dichroism spectrophotometry using an Aviv62DS spectropolarimeter with a 3 mm pathlength cuvette at 25°C. In all cases data was subtracted from the spectra of a solution containing only buffer (10 mM Tris-HCl, 80 mM KCl, pH 7.5). The circular dichroism thermal denaturation studies were carried out by scanning between 25-95°C with a temperature step of 1°C (temperature dead band of 0.10°C) and an equilibration time of 0.5 min. The data was collected over 2 seconds and the average value was recorded. UV-vis measurements were carried out using a 3 mm pathlength cuvette on an Agilent A453 spectrometer.

Ib. Synthesis of 5-[4-N(3-aminopropyl amidyl) phenyl]-15- Porphyrin (P)

To a solution of 5-(4-carboxyphenyl)-15-phenyl porphyrin\(^1\) (0.070 g, 0.138 mmol) in 10 ml of dry CH\(_2\)Cl\(_2\) was added oxalyl chloride (0.080 g, 0.62 mmol) and a catalytic amount of DMF (0.05 ml), and the mixture was stirred at room temperature overnight to generate the acid chloride. After evaporation of solvents in \textit{vacuo} the residue was redissolved in dry THF (5 ml) and a solution of n-1-Boc-1, 3 diaminopropane•HCl (0.061 g, 0.354 mmol) and DIPEA (0.10 g, 0.81 mmol) in dry CH\(_2\)Cl\(_2\) (5 ml) was added. The mixture was stirred at room temperature for 6hr. The solvent and excess reagents were evaporated and the crude product was taken up in 50 ml of CH\(_2\)Cl\(_2\), washed with 0.5 N NaOH (50 ml), 0.5 N HCl (50 ml) twice, brine (50 ml) twice, and dried over Na\(_2\)SO\(_4\). The organic layer was evaporated
and was purified by column chromatography (SiO$_2$, 5% MeOH/CH$_2$Cl$_2$) to give the fully protected product. Further treatment with 95% TFA/H$_2$O (5 ml) at room temperature for 4 hr followed by evaporation afforded crude porphyrin $P$ as dark green solid. The final chromatographic step was carried out by preparative HPLC (RP-C18; Gradient of 10% acetonitrile linearly increasing to 90% over 41 minutes, in 0.1%TFA/ water) to yield $P$ (63 mg, 80% over 2 steps).

m.p. decomposed at 292ºC; $^1$H NMR (500 MHz, DMSO-$d_6$) δ 9.01 (t, $J = 5.7$ Hz, 4H), 8.85 (s, 8H), 8.34 (d, $J = 8.5$ Hz, 8H), 8.30 (d, $J = 8.5$ Hz, 8H), 7.80 (s, b, 12H), 3.5 (m, overlap with water, 8H), 2.97 (hextet, $J = 6.9$ Hz, 8H), 1.94 (quintet, 6.9 Hz, 8H), -2.93 (s, 2H).

HR-ESI MS Calculated: C$_{36}$H$_{30}$N$_6$O [M+H]$^+$ 563.2559, Observed: 563.2578.

**Ic. Standard preparation of porphyrin conjugated oligonucleotides**

To a vial containing 2 mg of amine $P$ was added 1 ml of anhydrous DMF, and 50 µl anhydrous DIPEA. The resulting solution was transferred into a syringe and introduced into a cartridge containing reactant $N$-hydroxysuccinimide ester (NHS ester) modified oligonucleotide (1.0 µmol scale) tethered to a solid support. The amine solution was pushed through the cartridge five times and then the resin-linked DNA/amine mixture was agitated for 1 hr. This process was repeated three times. After which, the resin-linked DNA/amine mixture was agitated overnight. The amine solution was then removed and the cartridge was washed three times with 1ml aliquots of HPLC grade acetonitrile. The resin-linked DNA was dried by introducing argon flow through the cartridge for 1 hr. Cleavage
from the resin and global deprotection was achieved by introducing a 30% solution of NH₄OH (3 ml) overnight at 55°C.

Purification of porphyrin tethered oligonucleotides was achieved using a Varian PRP-1 reverse-phase HPLC column. Briefly, an oligonucleotide solution containing 0.1 M triethylamine acetate (TEAA) was heated at 95°C for 10 min, and then rapidly introduced into the HPLC injector. Throughout the run the column was maintained at 65°C using a heat jacket. The solvent system used is detailed in Table 1.

Preparation of the phenyl substituted ODNs 1Ph and 2Ph were carried out in a similar manner to the experimental section described above where 1.78 µl of 3-phenyl-1-propylamine was used as the amine reactant with the respective NHS ester modified oligonucleotides.

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**Table 1.** Typical HPLC eluent gradient used for the purification of porphyrin tethered ODNs. A: 0.1M TEAA, 5% ACN, B: 100% ACN.
The de-salted and HPLC purified samples were prepared for MALDI-TOF analysis using a 9:1:1 mixture of 2,4,6-trihydroxyacetophenone (THAP) (10 mg/ml in 50% acetonitrile/H$_2$O), ammonium citrate (50 mg/ml in H$_2$O), and oligonucleotide solution, respectively.

**Id. Experimental protocols for self-assembly of quadruplex DNA**

*i) Standard quadruplex formation:* Freshly prepared and purified ODNs were exposed to quadruplex forming conditions by diluting an appropriate aliquot of ODN (dissolved in water) into an eppendorf containing buffer (10 mM Tris-HCl, 80 mM KCl, pH 7.5). The final concentration of the ODN in the eppendorf tube was 25 µM (single strand). After fastening the cap and sealing with parafilm the tube was heated at 95 °C for 15 min, followed by slow cooling to room temperature and subsequent incubation at 4 °C for 48 hr. The formation of parallel quadruplexes was assessed by circular dichroism spectrophotometry.

*ii) Incubation of preformed quadruplex DNA with HP-$\beta$-CD:* To a solution of self-assembled quadruplex DNA (*vide supra*) was added 7.2% (w/v) HP-$\beta$-CD, such that the final concentration of ODN was 25 µM (single strand). The resulting solution was agitated for 48 hr at room temperature.

*iii) Formation of quadruplex DNA in the presence of HP-$\beta$-CD:* To a freshly prepared solution containing buffer (10 mM Tris-HCl, 80 mM KCl, pH 7.5) and ODN was added 7.2% (w/v) HP-$\beta$-CD. The final DNA concentration was 25 µM (single strand).
resulting mixture was heated at 95 °C for 15 min, followed by slow cooling to room temperature and subsequent incubation at 4 °C for 48 hr.

IIa. CD spectra of 2P and 2Ph after self-assembly

![Circular dichroism spectra of 2P and 2Ph after exposure to quadruplex self-assembly conditions.](image)

**Figure 1.** Circular dichroism spectra of 2P (○) and 2Ph (■) after exposure to quadruplex self-assembly conditions. All measurements were carried out in 80 mM KCl, 10 mM Tris-HCl (pH = 7.5). Concentrations of all ODNs were 25 µM (single strand).

In analogy with ODN 1P (cf. Figure 1, main text), ODN 2P (which also contains a porphyrin head group) self-assembles to form a parallel quadruplex under the experimental conditions outlined in section Idi. ODN 2Ph d(PhTG₄T₄) containing a phenyl head group does form some secondary structure. However, the profile is not indicative of a well-defined parallel quadruplex. These results show that the ODN series comprising four guanines in a row also require porphyrin-based aromatic interactions to self-assemble into a quadruplex, under the conditions of the experiment.
IIb. CD spectra of preformed quadruplex [1P]₄ before and after addition of HP-β-CD

![Graph showing CD spectra](image)

**Figure 2.** Full circular dichroism spectra of [1P]₄ prior to (○) and after (●) addition of HP-β-CD 7.2% (w/v). All measurements were carried out in 80 mM KCl, 10 mM Tris-HCl (pH = 7.5). Concentrations of all ODNs were 25 µM (single strand).

These results show that the porphyrin Soret region (380-440 nm) does not display any significant induced circular dichroism signal prior to addition of HP-β-CD. This observation, taken together with UV-vis data (cf. main text, Figure 3) is rationalized by the presence of substantial self-stacking interactions between the porphyrin chromophores which leads to a low intensity profile in the Soret region. In contrast, addition of HP-β-CD leads to an increase in intensity of a negative peak centered at 403 nm indicative of host-guest complexation.
IIc. CD and UV-vis spectra of preformed quadruplex [4P]₄ before and after addition of HP-β-CD

Figure 3. (A) Normalized electronic absorption and (B) circular dichroism spectra of [4P]₄ prior to (○) and after (●) addition of 7.2% (w/v) HP-β-CD. Measurements taken with HP-β-CD were carried out after a 48 hr incubation period. All measurements were carried out in 80 mM KCl, 10 mM Tris-HCl (pH = 7.5). Concentrations of all ODNs were 25 µM (single strand).

The UV-vis results clearly show that addition of HP-β-CD causes a decrease in the blue shifted shoulder of the normalized electronic absorption spectra of the porphyrin Soret band indicative of HP-β-CD forming inclusion complexes with the porphyrin head-groups of
[4P]₄. This binding event can also be observed by an increase in negative ellipticity at 403 nm. However, addition of HP-β-CD does not alter the circular dichroism signature in the ODN region. This latter finding supports the contention that, unlike for [1P]₄, capping with HP-β-CD does not have any significant effect on the stability of quadruplex [4P]₄ since the presence of six contiguous guanines is sufficient to form a stable quadruplex (cf. profile for [4]₄, main text, Figure 1).

IId. Temperature induced absolute change in ellipticity for quadruplexes [1P]₄, [4P]₄, non-aggregated ODN 1, and ODN 1P in the presence of HP-β-CD

![Figure 4](image_url)

**Figure 4.** Temperature induced change in ellipticity for quadruplexes [1P]₄ and [4P]₄ prior to (○ and ○, respectively) and after (● and ●, respectively) addition of 7.2% (w/v) HP-β-CD. Also shown are temperature induced change in ellipticity for control non-aggregated ODNs 1 (—), and 1P (◆) (where HP-β-CD is added to 1P prior to quadruplex assembly). Measurements were carried out at 267 nm for all the ODN samples containing parent sequence 1 and at 266 nm for ODNs containing parent sequence 4. All ODN solutions
were in 80 mM KCl, 10 mM Tris-HCl (pH = 7.5). Concentrations of all ODNs were 25 µM (single strand).

The above figure plots the absolute change in the maximum ellipticity for quadruplexes [1P]₄ and [4P]₄ as the temperature is increased from 25 to 95 °C. As can be seen from inspection of the graph, control quadruplex [4P]₄ shows only a small change in ellipticity with no saturation behavior even at 95 °C. These results suggest that [4P]₄ in potassium containing buffer is still substantially folded under the conditions of the denaturation experiment. Furthermore, thermal denaturation experiments of control ODNs that do not form quadruplexes under standard self-assembly condition (i.e., ODN 1, and ODN 1P incubated in the presence of HP-β-CD) show the expected thermal profile with insignificant change in ellipticity through the thermal denaturation scan.

IIc. CD spectra of ODNs 4P and 1P with HP-β-CD present during quadruplex self-assembly
**Figure 5.** Circular dichroism spectra of ODNs 4P (●) and 1P (◆) wherein 7.2% (w/v) HP-β-CD was added prior to quadruplex self-assembly. Measurements were carried out in 80 mM KCl, 10 mM Tris-HCl (pH = 7.5). Concentrations of all ODNs were 25 µM (single strand).

Addition of HP-β-CD prior to exposure of ODN 4P to quadruplex self-assembly conditions still resulted in the presence of a parallel quadruplex. This result is consistent with the notion that HP-β-CD does not adversely destabilize the formation of quadruplex [4P]₄.

**IIf. CD spectra of ODN 1 self-assembled under varying incubation time-frames**

![CD Spectra](image)

**Figure 6.** Circular dichroism spectra of ODN 1 (25 µM, single strand) incubated at 4°C for 60 hr (—), 108 hr (— —), 156 hr (— — —), and 276 hr (———), respectively. Measurements were carried out in 80 mM KCl, 10 mM Tris-HCl (pH = 7.5).
Under the standard incubation time frames used in this study (48 hr at 4°C) ODN 1 does not form a parallel quadruplex (see Figure 1, main text). After incubating for 276 hr (ca. 11.5 days at 4°C) ODN 1 displays a positive ellipticity at 257 nm (the maximum ellipticity is shifted ca. 10 nm from what is observed for parallel quadruplex 1P) and a negative peak at 237 nm suggesting the possible formation of a parallel quadruplex. However, even after such prolonged incubation times a substantial shoulder (centered at 275 nm) is visible. Thus, even after long incubation times quadruplex formation is not complete and other species may also be present in solution. These findings when compared with ODN 1P suggest that addition of the porphyrin head group gives kinetic stabilization to quadruplex formation since only 48 hr at 4°C is necessary for the self-assembly of 1P to [1P]₄.

IIIa. MALDI-TOF data for conjugated ODNs 1P and 2P

![MALDI-TOF data for conjugated ODNs 1P and 2P](image)
Figure 7. (A) MALDI-TOF data for ODN 1P \text{d(PTGGTTTT)} after HPLC purification. Calculated monoanion; 3239.77, observed; 3240.8. (B) MALDI-TOF data for ODN 2P \text{d(PTGGGTTTT)} after HPLC purification. Calculated monoanion; 3568.82, observed; 3569.8. (C) A representative illustration of ODN 1P.

IIIb. MALDI-TOF data for conjugated ODNs 1Ph and 2Ph
Figure 8. (A) MALDI-TOF data for ODN 1Ph d(PhTGGGTTTT) after HPLC purification. Calculated monoanion; 2812.62, observed; 2814.8. (B) MALDI-TOF Data for ODN 2Ph d(PhTGGGTTTT) after HPLC purification. Calculated monoanion; 3141.68, observed; 3139.5. (C) A representative illustration of ODN 1Ph.

IIIc. MALDI-TOF data for conjugated control ODNs 4P and SSP

Figure 9. (A) MALDI-TOF data for ODN 4P d(PTGGGGGTTTT) after HPLC purification. Calculated monoanion; 4226.92, observed; 4229.3. (B) MALDI-TOF data for ODN SSP d(PCGTCATATCTTA) after HPLC purification. Calculated monoanion; 4387.97, observed; 4390.6.

References