Assembly of an Antiparallel Homo-Adenine DNA Duplex by Small Molecule Binding

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Supporting Information

Materials and Methods:

Sample Preparation. $(dA)_4$ and 3'-$d(A)_8$-5'-5'-$d(A)_8$-3' oligodeoxynucleotides were synthesized in house on an automated synthesizer using standard phosphoramidite chemistry**.** $(dA)_8$, $(dA)_{16}$, $(dA)_{32}$, d(GACCCGCA_8CCTCGCC) and d(GGCGAGGA_8GCGGGTC) oligonucleotides were purchased from IDT (Coralville, IA). Full-length oligonucleotides were separated from failure sequences by denaturing polyacrylamide gel electrophoresis. Full-length products were extracted from the gel matrix using the crush-and-soak method followed by ethanol precipitation and desalting by passage over a 1 m G-25 sephadex column. Column fractions containing purified oligonucleotides were pooled, lyophilized and resuspended in dH_2O. Coralyne chloride and proflavine hemisulfate were purchased from Sigma and used without further purification.

Oligonucleotide and small molecule concentrations were determined by UV-Vis spectroscopy using the following extinction coefficients: $(dA)_4$, ε_{260} = 51 400 M^{-1} cm^{-1}; $(dA)_8$, ε_{260} = 99 400 M^{-1} cm^{-1}; $(dA)_{16}$, ε_{260} = 195 400 M^{-1} cm^{-1}; $(dA)_{32}$, ε_{260} = 387 400 M^{-1} cm^{-1}; GACCCGCA_8CCTCGCC, ε_{260} = 212 100 M^{-1} cm^{-1}; and GGCGAGGA_8GCGGGTC, ε_{260} = 231 900 M^{-1} cm^{-1}; 3'-$d(A)_8$-5'-5'-$d(A)_8$-3', ε_{260} = 195 400 M^{-1} cm^{-1}; coralyne chloride, ε_{420} = 14 500 M^{-1} cm^{-1}; proflavine hemisulfate, ε_{444} = 38 900 M^{-1} cm^{-1}.

Polyacrylamide gel electrophoresis (PAGE). Oligonucleotides were 5'-end labeled using γ-^{32}P-ATP (ICN) and T4 polynucleotide kinase (New England Biolabs). Non-denaturing PAGE experiments were run in a standard 1× TBE buffer (Tris-Borate-EDTA), at a constant power of 7 W and an ambient temperature of 4°C. Gels were imaged using a Fuji Phosphor Imager (FLA-3000).

Circular dichroism (CD) and UV-Vis spectrophotometry. CD spectra were acquired on a JASCO J-810 CD spectropolarimeter equipped with Peltier temperature control unit. Spectra were acquired using a 1 cm path length cell. UV-Vis absorbance measurements were performed using a HP 8453 UV-Vis diode array spectrophotometer equipped with an Agilent 89090A Peltier temperature control unit. UV melting profiles were acquired by increasing the sample temperature at a rate of 1°C min^{-1} from 5 to 80°C.

Atomic Force Microscopy (AFM). Scanning force images were acquired using a Nanoscope IIIa AFM (Digital Instruments) equipped with a J scanner operating in tapping mode. AFM tips were NSC12 non-contact silicon rectangular cantilevers (Mikromasch USA, Portland), which were cleaned with ozone prior to use. Samples (20 μl) were deposited onto freshly cleaved mica, incubated for 30 min at 4°C, rinsed once with dH_2O (4°C), wicked dry by touching an edge of the mica to filter paper, blown dry with nitrogen gas and stored overnight in a vacuum desiccator at room temperature. Samples were imaged under ambient conditions. Images were flattened to remove background slope in the horizontal dimensions.

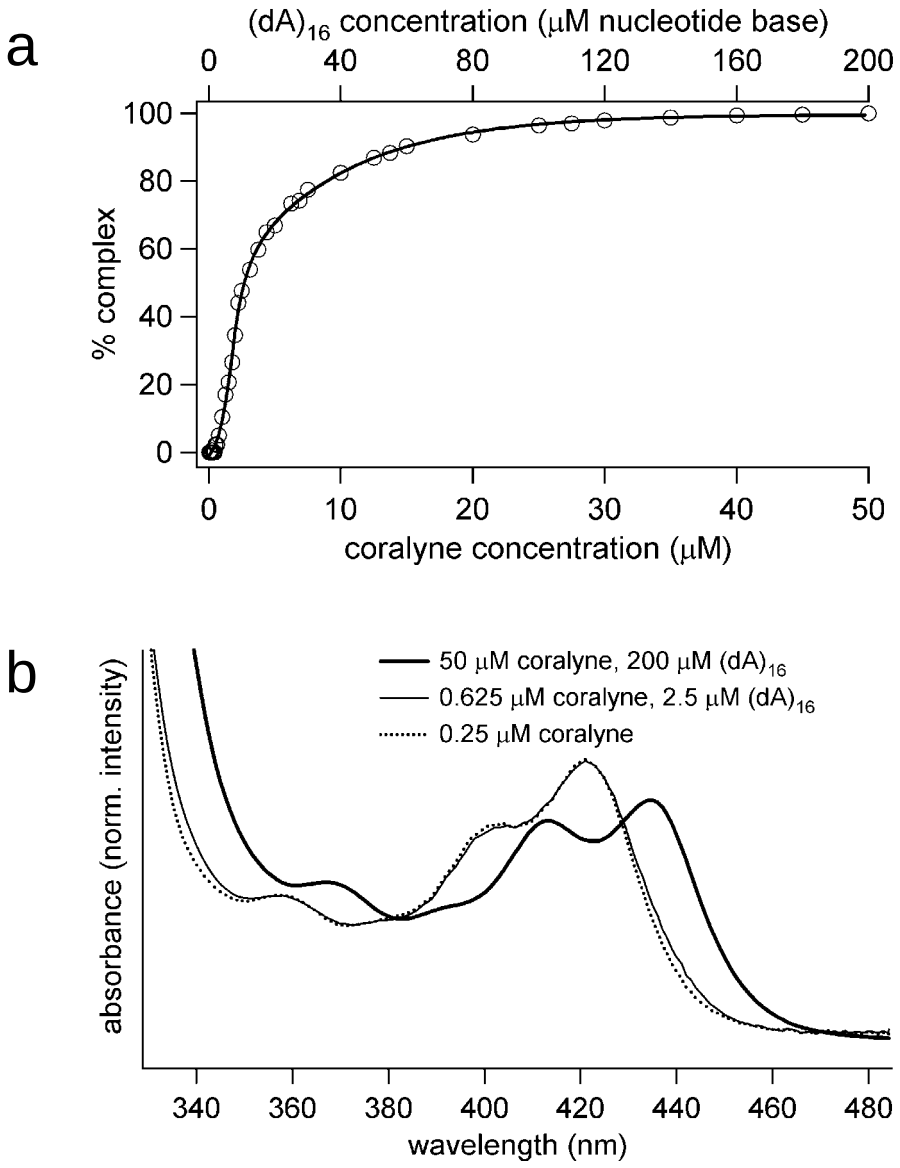

Figure S1. **(a)** Plot of percent $(dA)_{16}$–coralyne complex formed as a function of DNA and coralyne concentration. The relative concentration of coralyne to $(dA)_{16}$ was one coralyne molecule per four nucleotide base for all data points, the stoichiometry previously determined for the poly(dA)–coralyne complex (Polak and Hud, *Nucleic Acids Res.* **2002**, 30, 983-992). Percent of $(dA)_{16}$–coralyne complex formed at each concentration was determined by performing a least-squares fit of the corresponding UV absorption spectrum as a weighted sum of two absorption spectra, which were the spectrum of 200 μM $(dA)_{16}$ (in nucleotide base), 50 μM coralyne and a spectrum of 0.025 μM coralyne (i.e. free coralyne with no DNA). All samples contained 115 mM NaCl and 13 mM NaCacodylic, pH 6.8. Spectra were acquired at 22°C. **(b)** UV absorbance spectra representative of those used to generate the plot in **a**. The spectral region from 340 to 375 nm was that used to determine the fraction of $(dA)_{16}$–coralyne complex formed.

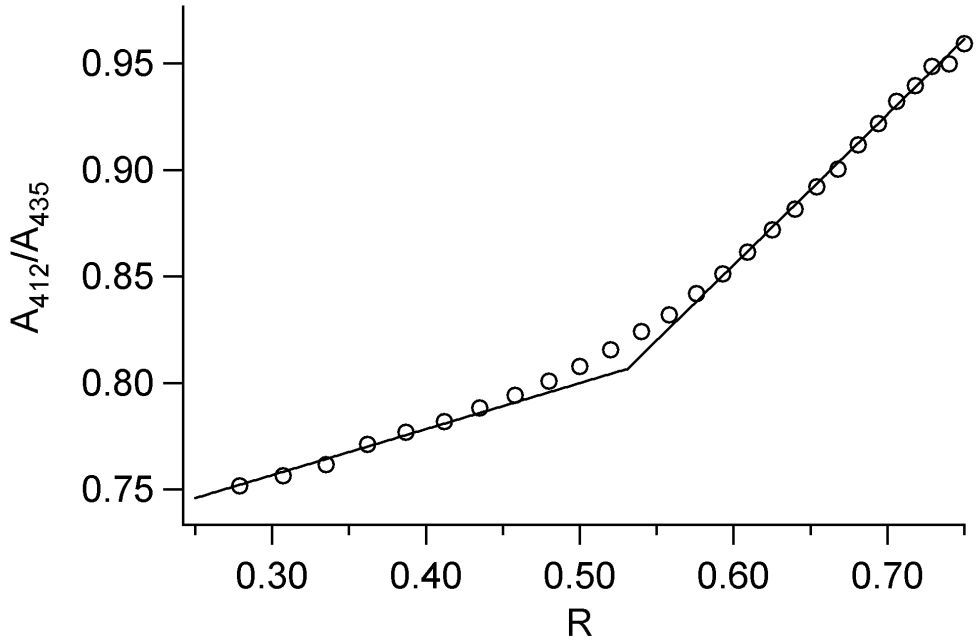

Figure S2. Job Plot analysis of **plusA8·minusA8** with coralyne. A_{412}/A_{435} is the ratio of coralyne absorbance at 412 nm versus 435 nm. R = [coralyne]/([coralyne] + [(dA)/4]). The combined concentration of coralyne and (dA)/4 was 15 μM for each data point in the Job plot.

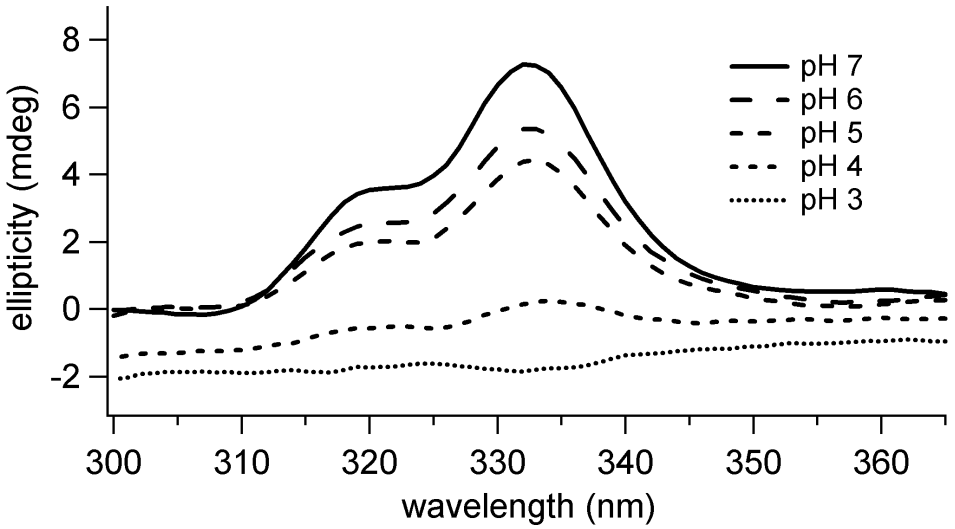

Figure S3. The 300 to 365 nm region of CD spectra of $(dA)_{32}$ with coralyne at various pH values. The reduction of the positive CD bands in this region at lower pH indicates the reduced stability of the $(dA)_{32}$–coralyne complex with decreasing pH. Samples were 55 μM nucleotide base, 14 μM coralyne, 115 mM NaCl and 13 mM NaCacodylic. Samples with pH lower than 7 were prepared by the titration of a pH 7 sample with 1M HCl.