SUPPORTING INFORMATION

Helix-forming Properties of Size-expanded DNA (xDNA), an Alternative Four-base Genetic Form

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Figure S1. Thermal denaturation curves of xDNA complexes and control DNA duplexes measured at total oligomer concentration of 5.0 µM in a buffer containing 10 mM MgCl₂, 100 mM NaCl, and 10 mM Na-PIPES, pH 7.0. DNA samples were monitored at 260 nm and xDNA samples were monitored at 320 nm. (A) xDNA sequence 1* and DNA sequence a. (B) xDNA sequences 2:3 and DNA sequences b:c. (C) xDNA sequences 4:5 and DNA sequences d:e. (D) xDNA duplex of 6:7 and triplex 6:7:6 and DNA duplex f:7. (E) Sequence xA₅T₅. (F) Sequence xT₅A₅. Curves in A-D were fit well using a two-state approximation, while complexes in E and F appeared to show non-two-state behavior.

* see Figure 1 in the manuscript for sequence numbers.
**Figure S2.** Binding stoichiometry of sequences 2 and 3 estimated by gel shift (20% native polyacrylamide gel in 1x TBE buffer). Each lane was loaded with 5 µL total of oligomers 2 and 3 at 200 µM in the same buffer as described in Figure S1. Ratios are shown at the bottom of each lane.
Figure S3. Fluorescence changes for xDNA. (A) Fluorescence spectra of ss 2, ss 3 and ds 2:3 excited at 320 nm, measured in the same buffer as for T_m studies. (B) Fluorescence spectra of ss 6, ds 6:7 in PIPES or Tris buffer excited at 320 nm.
**Figure S4.** Fluorescence spectra excited at 320 nm of single strand 4, single strand 5 and duplex 4:5 at 5.0 µM in same buffer as for $T_m$ measurements (see Figure 1).
**Figure S5.** CD spectra of xDNA ss oligomers and helical complexes. (A) 2 and 3. (B) 4 and 5. (C) 6 and 7. Conditions: 100 mM NaCl, 10 mM MgCl2, 10 mM Na·PIPES, pH=7.0, 5.0 µM DNA.