SUPPORTING INFORMATION

Expanded-size Bases in Naturally-sized DNA:

Evaluation of Steric Effects in Watson-Crick Pairing

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CONTENTS:

Oligodeoxynucleotide synthesis and characterization. 5'-O-tritylated phosphoramidite analogues of nucleosides were synthesized as described.[1] Oligodeoxynucleotides were synthesized on 1.0 μmole scale on an Applied Biosystems 394 synthesizer using standard β-cyanoethyl phosphoramidite chemistry, but with extended (120 s) coupling times for modified nucleosides. Stepwise coupling yields for nonnatural nucleosides were all greater than 95% as determined by trityl cation monitoring. All oligomers were deprotected in concentrated ammonium hydroxide (55 ^{o}C, 16 h), purified by preparative 20% denaturing polyacrylamide gel electrophoresis, and isolated by excision and extraction from the gel, followed by dialysis against water. The recovered material was subsequently quantified by absorbance at 260 nm with molar extinction coefficients determined by the nearest neighbor method.[2] Values for oligomers containing modified nucleotides were estimated by measuring the molar extinction coefficients of modified nucleosides at 260 nm and adding these values to the calculated values of natural DNA fragments. Modified oligonucleotides in the study were characterized by ESI or MALDI-TOF mass spectrometry (Table S1).

Table S1. Mass spectrometry data for oligonucleotides containing expanded bases xT, xA, or F_o.

	Oligodeoxynucleotide	Calculated	Observed
1	5'-CTT TTC **xT**TT CTT-3'	3591.6	3592.0
2	5'-AAG AA**xT** GAA AAG-3'	3786.6	3789.0
3	5'-**xT**CG CGC G-3'	2148.4	2148.9
4	5'-CTT TTC **xA**TT CTT-3'	3604.4	3606.8
5	5'-AAG AA**xA** GAA AAG-3'	3796.5	3798.1
6	5'-AAG A**xAxA** GAA AAG-3'	3846.5	3845.2
7	5'-AAG **xAxAxA** GAA AAG-3'	3896.5	3895.7
8	5'-**xA**CG CGC G-3'	2158.4	2158.0
9	5'-**xA**CG CGC GT-3'	2462.6	2464.3
10	5'-CTT TTC **F_o**TT CTT-3'	3464.6	3467.6

Thermal denaturation methods. Solutions for the thermal denaturation studies contained either a 1:1 ratio of two complementary oligomers or only a self-complementary oligomer. After solutions were prepared, they were heated to 95 °C for 10 min and allowed to cool to room temperature slowly for at least 2 h, then cooled at 5 °C for at least 4 h. Melting studies were carried out in Teflon-stoppered 1 cm path length quartz cells (under nitrogen atmosphere when temperature was below 20 °C) on a Varian Cary 1 UV-Vis spectrophotometer equipped with a thermoprogrammer and Peltier temperature control. Absorbance was monitored while temperature was changed at a rate of 0.5 °C/min. Melting plots were essentially the same for heating or cooling at this rate. Experiments were monitored at 260 nm. In most cases the complexes displayed apparent two-state transitions, with all-or-none melting curves from bound duplex to free single strands. Computer fitting of the melting data using Meltwin 3.0b provided both melting temperatures T_m and free energy values for the complexes. The thermodynamic parameters were also calculated from van't Hoff plots by plotting $1/T_m$ vs. ln(C/4); in most cases close agreement was observed, indicating that the two- state approximation is

Table S2. Free Energies($-\Delta G°_{37}$ (kcal/mol)) and Melting Temperatures(T_m(°C)) for DNA Duplexes Containing F_o placed opposite other bases (X).

	X-F_o	T_m(°C)[a]	$-\Delta G°_{37}$ (kcal/mol)[b] van't Hoff	$-\Delta G°_{37}$ (kcal/mol)[c] curve fits	$-\Delta G°_{37}$(kcal/mol)[d] average
1	xA-F_o	29.1	6.5±0.3	6.7±0.1	6.6±0.2
2	A- F_o	19.7	4.2±0.3	4.8±0.2	4.5±0.2
3	G- F_o	21.5	5.4±0.4	5.4±0.2	5.4±0.3
4	T- F_o	15.0	3.7±0.8	4.2±0.6	4.0±0.7
5	C- F_o	17.7	4.0±0.5	4.3±0.2	4.2±0.4

[a]Conditions: 100 mM NaCl, 10 mM $MgCl_2$, 10 mM Na•PIPES, pH=7.0, 5.0 μM DNA, with DNA sequences AAG AA**X** GAA AAG and CTT TTC **F_o**TT CTT. [b]Obtained by plotting 1/ T_m vs ln(C_T) with data from five concentrations; standard deviations are shown. [c]Average from five denaturation curves; standard deviations are shown. [d]Average of ΔG° obtained by van't Hoff analysis and curve fits.

reasonable for these specific sequences. T*m* and ΔG° values of expanded base pairs and mismatches are summarized in Table 1 (main text). Data for F_o containing base pairs are summarized in Table S2 here.

CD spectroscopy. Circular dichroism spectra were measured on a Jasco J-810 spectropolarimeter between 400 and 220 nm in a standard buffer containing 100 mM NaCl, 10 mM $MgCl_2$, 10 mM Na•PIPES (pH 7.0). DNA duplexes were at 5.0 μM. Spectra were acquired every 1 nm with a bandwidth setting of 1 nm at a speed of 50 nm/min, averaging 6 scans. Raw CD amplitude data in mdeg were converted into Δε using established methods.[3]

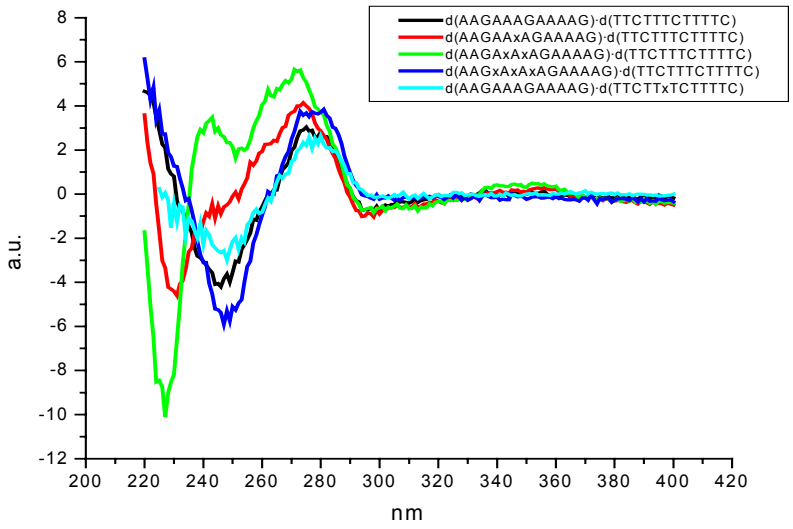

Figure S1. CD spectra of DNA duplexes containing expanded bases or base pairs. All samples were 5 μM concentration, dissolved in a buffer containing 100 mM NaCl, 10 mM $MgCl_2$ and 10 mM Na•PIPES (pH=7.0).

Fluorescence experiments. Steady-state fluorescence measurements were carried out on a Spex Fluorolog 3 fluorescence spectrometer equipped with Lauda Brinkmann RM 6 temperature controller. Quantum yields (Φ_f) were calculated with Fluorescein in 0.1N NaOH solution as a reference. Quantum yields were summarized in Table S3.

Table S3. Fluorescence properties of DNAs containing size-expanded nucleobases.

	Sequence	Temperature	Quantum yield (Φ_f)
1	CTTTTC**x**A**T**TCTT	15°C	0.20
2	AAGAA**x**A**G**AAAAG	15°C	0.04
3	CTTTTC**x**T**T**TCTT	15°C	0.33
4	AAGAA**x**T**G**AAAAG	15°C	0.01
5	(AAGAA**T**GAAAAG)•(CTTTTC**x**A**T**TCTT)	80°C	0.16
6	(AAGAA**T**GAAAAG)•(CTTTTC**x**A**T**TCTT)	15°C	0.06
7	(AAGAAA**G**AAAAG)•(CTTTTC**x**A**T**TCTT)	15°C	0.10
8	(AAGAA**G**GAAAAG)•(CTTTTC**x**A**T**TCTT)	15°C	0.11
9	(AAGAA**C**GAAAAG)•(CTTTTC**x**A**T**TCTT)	15°C	0.10
10	(AAGAAϕGAAAAG)•(CTTTTC**x**A**T**TCTT)	15°C	0.09
11	(AAGAAA**G**AAAAG)•(CTTTTC**x**T**T**TCTT)	80°C	0.29
12	(AAGAAA**G**AAAAG)•(CTTTTC**x**T**T**TCTT)	15°C	0.06
13	(AAGAA**T**GAAAAG)•(CTTTTC**x**T**T**TCTT)	15°C	0.05
14	(AAGAA**G**GAAAAG)•(CTTTTC**x**T**T**TCTT)	15°C	0.07
15	(AAGAA**C**GAAAAG)•(CTTTTC**x**T**T**TCTT)	15°C	0.07
16	(AAGAAϕGAAAAG)•(CTTTTC**x**T**T**TCTT)	15°C	0.09
17	xACGCGCG• xACGCGCG	80°C	0.12
18	xACGCGCG• xACGCGCG	15°C	0.02
19	xTCGCGCG• xTCGCGCG	80°C	0.12
20	xTCGCGCG• xTCGCGCG	15°C	0.03

Samples 1-16 were dissolved in the buffer of 100 mM NaCl, 10 mM $MgCl_2$, 10 mM Na•PIPES, pH=7.0. Samples 17-20 were prepared in 1 M NaCl, 10 mM NaH_2PO_4, pH=7.0. Spectra were recorded with excitation at 320 nm. Quantum yields were calculated with fluorescein in 0.1 N NaOH aqueous solution as a standard.

1. (a) Liu, H.; Gao, J.; Maynard, L.; Saito, D.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, *126*, 1102-1109. (b) Guy, A.; Duplaa, A. M.; Ulrich, J.; Teoule, R. *Nucleic Acids Res.* **1991**, *19*, 5815-5820.
2. Borer, P. N. In *Handbook of Biochemistry and Molecular Biology*, 3rd ed.; Fasman, G. D., Ed.; CRC Press: Cleveland, **1985**; Vol. I, p. 589.
3. Berova, N.; Nakanishi, K.; Woody, R.W. (Eds.) *Circular Dichroism: Principles and Applications* 2nd Ed.; Wiley: New York, 2000, pp. 29-33.