Effect of the Universal Base 3-Nitropyrrole on the Selectivity of Neighboring Natural Bases

Supplementary information:

EXPERIMENTAL

Preparation of oligonucleotides: Oligonucleotides were synthesized by The Midland Certified Reagent Company using solid phase phosphoramidite monomers. Oligonucleotides were purified by gel filtration and lyophilized prior to shipping. Synthesis was checked by electrospray mass spectrometry. Upon receipt, oligonucleotides were dissolved in 1 x PE buffer (10 mM NaH_2PO_4, 0.1 mM EDTA, pH 7.0) to make a stock solution. The concentration of the stock solutions was determined by measuring the absorbance of a diluted aliquot at 260 nm. Extinction coefficients were calculated from nearest neighbor parameters.[1]

Thermal dissociation (T_m) measurements: Ultraviolet spectra were recorded on a Beckman DU 640 spectrophotometer fitted with a six cell Peltier device and transport. Melting transitions were measured at 260 nm in 1 x PES buffer (10 mM NaH_2PO_4, 0.1 mM EDTA, 1M NaCl, pH 7.0), at a total oligomer strand concentration of 4uM. Absorbance versus temperature was obtained at a heating rate of 0.5°C/min for each duplex and at a total concentration of 4uM.

The melting temperature was determined by fitting the absorbance data as described.[2] This involves fitting the melting curves as sums of temperature dependent extinction coefficients for duplex and single stranded forms. The temperature at which half of the total DNA strands are in the duplex form is defined as the melting temperature.

Data

Control Duplexes (**3** + **4** and **6** + **7**)

GTCCTCC	**A**	**A**	TACGATG	Tm-Fit
	T	A		54.3
	T	G		57.3
	T	C		52.9
	T	T		61.9

1. Cantor, C.; Warshaw, M.M. Biopolymers 1970, 9, 1059-1077.
2. McDowell, J.A.; Turner, D.H. *Biochemistry* **1996**, *35*, 14077-14089.

A	T	54.5
G	T	56
C	T	53.9
T	T	62.7

GTCCTCC **G** A TACGATG

A	T	56
G	T	55.4
C	T	64.8
T	T	57

C	A	57.6
C	G	61.8
C	C	54.9
C	T	65.9

GTCCTCC **C** **A** TACGATG

G	A	55
G	G	59
G	C	55.4
G	T	63.7

A	T	52.7
G	T	63.3
C	T	51.4
T	T	53.9

GTCCTCC **T** **A** TACGATG

A	A	53.9
A	G	57.3
A	C	53.3
A	T	60.9

A	T	60.9
G	T	56.9
C	T	53.6
T	T	56.3

GTCCTCC **A** **G** TACGATG

T	A	57.7
T	G	58.4
T	C	63.6
T	T	56.7

A	C	56.9
G	C	58.4
C	C	53.9
T	C	63.5

GTCCTCC **A** **C** TACGATG

T	A	52.8
T	G	64
T	C	51.1
T	T	52.8

A	G	55
G	G	60
C	G	56.4

T	G	65

GTCCTCC	**A**	**T**	TACGATG

A	A	53
G	A	58.3
C	A	53.2
T	A	62.8

T	A	61.7
T	G	56.2
T	C	54.4
T	T	55.5

3-Nitropyrrole Duplexes (**1** + **2** and **5** + **2**)

GTCCTCC	**G**	*	TACGATG	Fit

A	A	54.5
G	A	50.6
C	A	56.1
T	A	51.8
A	G	54.3
G	G	51.2
C	G	54.9
T	G	52
A	C	54.2
G	C	51.6
C	C	52.8
T	C	51.2
A	T	54.8

	G	T		51.4
	C	T		53.8
	T	T		50.6

GTCCTCC **A** * TACGATG

	A	A		50.1
	G	A		50.1
	C	A		49.6
	T	A		53.9
	A	G		50.2
	G	G		49.5
	C	G		49.7
	T	G		53.6
	A	C		49.3
	G	C		48.1
	C	C		45.8
	T	C		52.2
	A	T		49.3
	G	T		50.4
	C	T		49.4
	T	T		52.4

GTCCTCC **T** * TACGATG

	A	A		53
	G	A		49.8
	C	A		49.2
	T	A		50.7
	A	G		51.4

G	G	49.5
C	G	49
T	G	50.6
A	C	52
G	C	49.5
C	C	45.2
T	C	48.8
A	T	51.6
G	T	49.2
C	T	47
T	T	48.4

GTCCTCC **C** * TACGATG

A	A	49.3
G	A	54.4
C	A	47.8
T	A	48.5
A	G	48.9
G	G	52.4
C	G	50.2
T	G	50.1
A	C	48.7
G	C	52.6
C	C	44.7
T	C	46.3
A	T	48.8
G	T	53.4
C	T	48

	T	T		47.9

GTCCTCC	*	**G**	TACGATG	
	A	A		48.8
	A	G		48.4
	A	C		57.2
	A	T		51.1
	G	A		49.3
	G	G		49.3
	G	C		55.4
	G	T		50.1
	C	A		51.1
	C	G		50.2
	C	C		54.5
	C	T		50.8
	T	A		49.4
	T	G		49.2
	T	C		56
	T	T		50.5

GTCCTCC	*****	**A**	TACGATG	
	A	A		48.2
	A	G		50.3
	A	C		47.8
	A	T		53.8
	G	A		47.8

G	G	50.1
G	C	46.3
G	T	53.8
C	A	47.2
C	G	49.1
C	C	44.7
C	T	53.2
T	A	49.9
T	G	51.4
T	C	48.7
T	T	54.2

GTCCTCC ***** **T** TACGATG

A	A	54.6
A	G	50.5
A	C	48.9
A	T	50.5
G	A	52.8
G	G	48.2
G	C	46.8
G	T	49.9
C	A	52.2
C	G	48.1
C	C	45.4
C	T	49
T	A	53.7
T	G	48.5
T	C	47.6
T	T	50.4

GTCCTCC ***** **C** TACGATG

A	A	47.4
A	G	55.2
A	C	47.2
A	T	48.4
G	A	49.2
G	G	54.7
G	C	48.1
G	T	49.1
C	A	47.6
C	G	55.1
C	C	44.3
C	T	48.1
T	A	48.2
T	G	54.9
T	C	46.5
T	T	48.5