Supporting Information:

Table 1: Characteristics of the 6MAP-containing oligonucleotides

Sequence name	Local environment[1]	SS Quantum Yield[2]	Duplex Quantum Yield[2]	ΔT_m (°C)[3]	$\Delta\Delta H$ (kcal/mole)[4]
A3-5	AFA	0.017	0.013	5.7	5.5
A4-8	AFA	0.043	0.010	5.9	5.2
T3-8	AFA	0.022	0.012	0.7	5.5
T4-12	AFA	0.040	0.004	2.3	5.0
A3-6	AFT	0.036	0.019	2.2	3.6
A4-9	AFT	0.051	0.008	2.5	3.4
A4-19	AFT	0.079	0.014	2.7	3.0
T3-7	TFA	0.033	0.027	7.8	3.4
T4-13	AFC	0.068	0.020	2.0	2.4
AT-6	TFT	0.039	0.015	1.1	2.5
AT-12	TFT	0.030	0.010	2.8	1.8

[1]Sequences are reported in the 5' to 3' direction. [2]Measured relative to 6MAP monomer, which has a quantum yield of 0.34 relative to quinine sulfate (*1*). [3]Determined by UV absorption: $\Delta T_m = T_{mControl} - T_{m6MAP\ sequence}$ [4]Determined from fits to the UV melting profiles as described in the Materials and Methods: $\Delta\Delta H = \Delta H_{Control} - \Delta H_{6MAP\ sequence.}$

Figure 1:

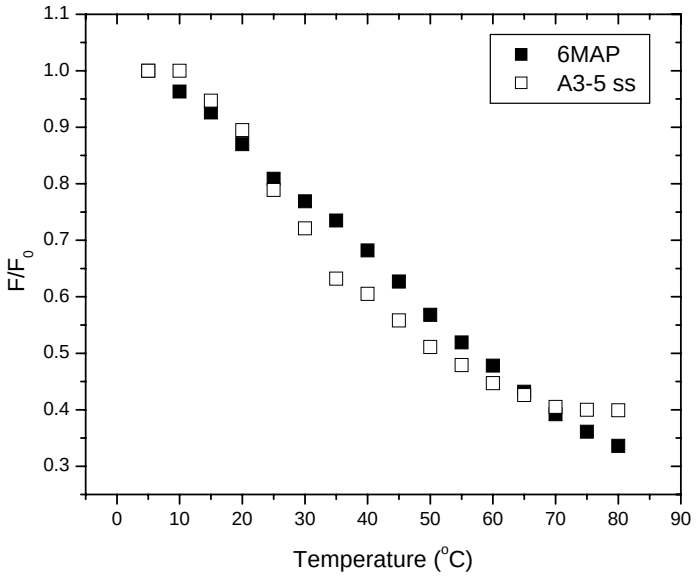


Figure 1: Fluorescence intensity of the 6MAP monomer and the A3-5 oligonucleotide as a function of temperature. Similar behavior is observed for the other 6MAP containing oligonucleotides. Fluorescence intensity is shown referenced to the first point (F_0) In general, the fluorescence intensity decreases linearly with increasing temperature.

Figure 2:

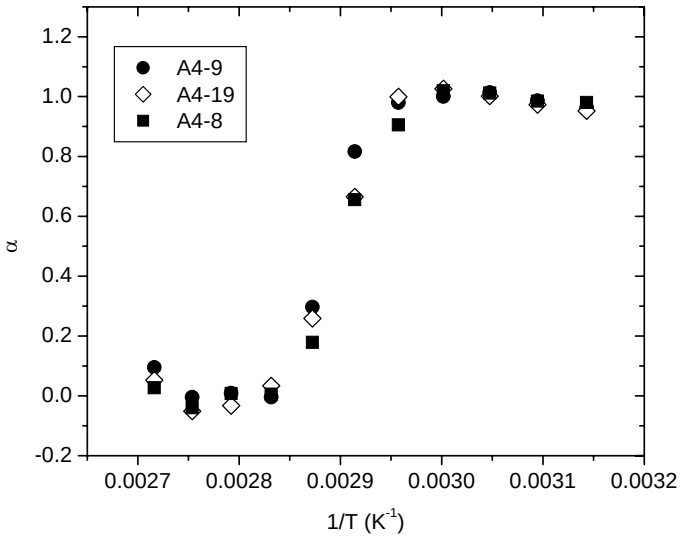
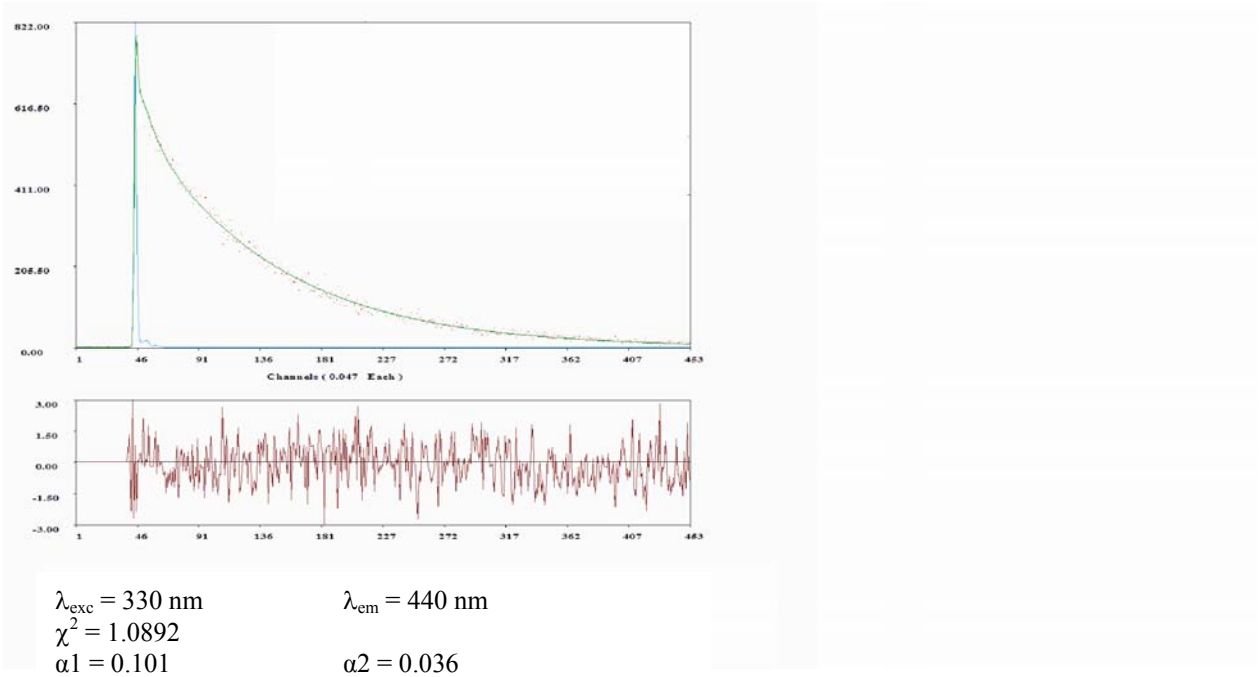


Figure 2: Fraction of single strands in the duplex state (α) as a function of temperature for the A4-8 (■), A4-9 (♦) and A4-19 (◊) duplexes. The duplex T_ms of all three duplexes are 71 ± 1 °C. Measurements were performed in 0.3 M NaCl with 0.05 M Mg^{2+} in a 10 mM Tris buffer at pH 7.4.

Figure 3:



λ_{exc} = 330 nm λ_{em} = 440 nm
χ^2 = 1.0892
$\alpha 1$ = 0.101 $\alpha 2$ = 0.036
$\tau 1$ = 4.59 ns $\tau 2$ = 0.39 ns

Figure 3: Representative fluorescence decay and fit. Fluorescence decay of the A3-5 duplex (dots) with associated fit (green solid line) and instrument response function (blue). Residuals derived from the fit are shown in brown underneath. Fluorescence decay was fit to a sum of exponentials (*2*) and fitting parameters are shown in the figure. Similar decays and fits were obtained for the other dodecamer duplexes.

Figure 4:

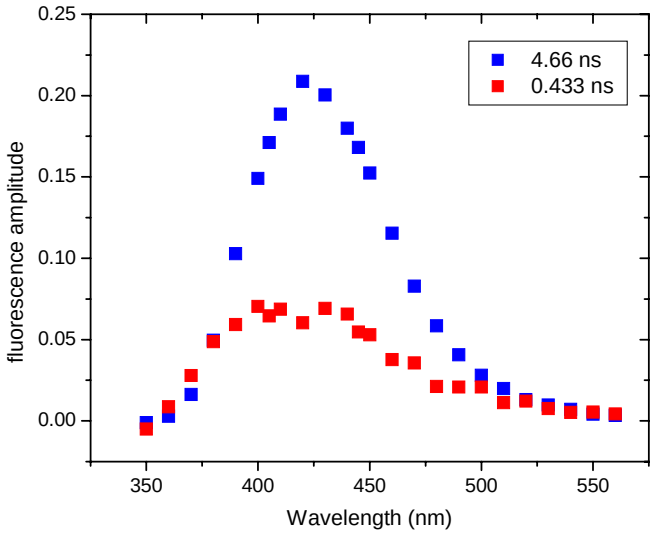


Figure 4: Decay-associated spectrum derived from the simultaneous global analysis of fluorescence decays (shown above) of the A3-5 duplex. Global analysis of the fluorescence decays was performed as described (*2*). The decay-associated spectrum illustrates that the 0.433 ns component peaks at shorter wavelength relative to the 4.66 ns component.

(1) Hawkins, M. E., Pfleiderer, W., Jungmann, O., and Balis, F. M. (2001) Synthesis and Fluorescence Characterization of Pteridine Adenosine Nucleoside Analogs for DNA Incorporation. *Anal. Biochem. 298*, 231-240.

(2) Knutson, J. R., Beechem, J. M., and Brand, L. (1983) Simultaneous Analysis of Multiple Fluorescence Decay Curves: A Global Approach. *Chem. Phys. Lett. 102*, 501-507.