Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Small molecule-mediated duplex formation of nucleic acids with 'incompatible' backbones

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Materials and Methods

Materials HPLC purified DNA and RNA oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA). 2',5'-RNA phosphoramidites were purchaed from ChemGenes (Wilmington, MA). UNA phosphoramidites were purchased from Link Technologies (Bellshill, Scotland). 2',5'-RNA and UNA were synthesized on an automated Expedite synthesizer using standard phosphoramidite chemistry with modified coupling times (30 min for 2',5'-RNA and 5 min for UNA). Post synthesis, 2',5'-RNA and UNA oligonucleotides were deprotected using standard protocols, purified by IE-HPLC (Dionex DNAPac PA-100), and desalted on a Sep-Pak Plus C18 cartridge. isoGNA phosphoramidite and oligonucleotide synthesis was performed as previously reported by Karri et. al. (ref. 10). Proflavine hemisulfate (Sigma) was used as received and dissolved in dH_2O. The extinction coefficient used to determine stock solution concentration was $\varepsilon_{444} = 38\ 900\ M^{-1}\ cm^{-1}$.

Thermal denaturation studies UV-vis spectra were acquired on a Cary 50 UV-Vis spectrophotometer equipped with a LC 600 Quantum Northwest Peltier temperature controller. Solutions contained 5 μM of each oligonucleotide for non-self-complementary sequences or 10 μM of oligonucleotide for self-complementary sequences (90 μM bp), and 45 μM proflavine when noted, all solutions contained 1 M NaCl (or 100 mM NaCl when noted), 0.1 mM EDTA, and 10 mM Na_2HPO_4 (pH 7.0). Spectra were measured using a 2 mm quartz cuvettes unless otherwise noted. For each experiment, two heating and two cooling temperature ramps (of 0.4 °C min^{-1}) were performed from 0 °C to 65 °C, or from 0 °C to 80 °C, and the absorbance intensity from 190-600 nm was recorded. Thermal melting profiles were produced from the resulting spectra by plotting A260 nm (baseline corrected) as a function of temperature. The heating and cooling T_m for each duplex was determined by taking the first derivative of the UV melting profiles. The amount of change in UV absorbance observed upon duplex melting (i.e., hyperchromicity) for the different duplexes of this study is, as expected, variable given that both the degree of base stacking in the duplex state and the degree of intra-strand base stacking in the single stranded state, which will be particular to each pairing system, contribute to the hyperchromicity associated with duplex melting. Thermal melting profiles were also collected for each individual nucleic acid strand of this study in the presence of proflavine. In some cases a cooperative transition was observed, indicating a self-structure of the nucleic acid with proflavine. However, when the complementary strand was present in the same sample the cooperative melting transition of the duplex was always observed at a higher temperature than the self-structure of the individual strand. Additionally, differences in the longest wavelength absorption bands of proflavine in the presence of the individual strands versus the complementary strands also indicated duplex formation.

Dilution studies Dilution studies were performed on an Agilent 8453 UV-vis spectrophotometer equipped with an Agilent 89090A Peltier temperature controller. Typically, samples initially contained 5 μM of each oligonucleotde (90 μM bp), 45 μM proflavine, and buffer solution (1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0). Dilutions were performed at 4 °C by adding the buffer solution incrementally to each sample. The UV-vis absorption profile was aquired after each addition of buffer. In order to maintain an absorbance between 1.2 and 0.1 AUs, quartz cuvettes from 1 to 10 cm were employed. Spectra were normalized and the change in proflavine absorbance, which shifted upon dissociation of proflavine from the oligonucleotide duplex, was used to determine proflavine association constants (K_a).

Circular Dichroism CD spectra were acquired on a JASCO J-810 CD spectropolarimeter equipped with a NESLAB temperature controller. CD spectra (220 to 600 nm) were measured at either 4 °C or 20 °C, as indicated, using a 1 cm path-length quartz cuvette.

Suplementary Figures

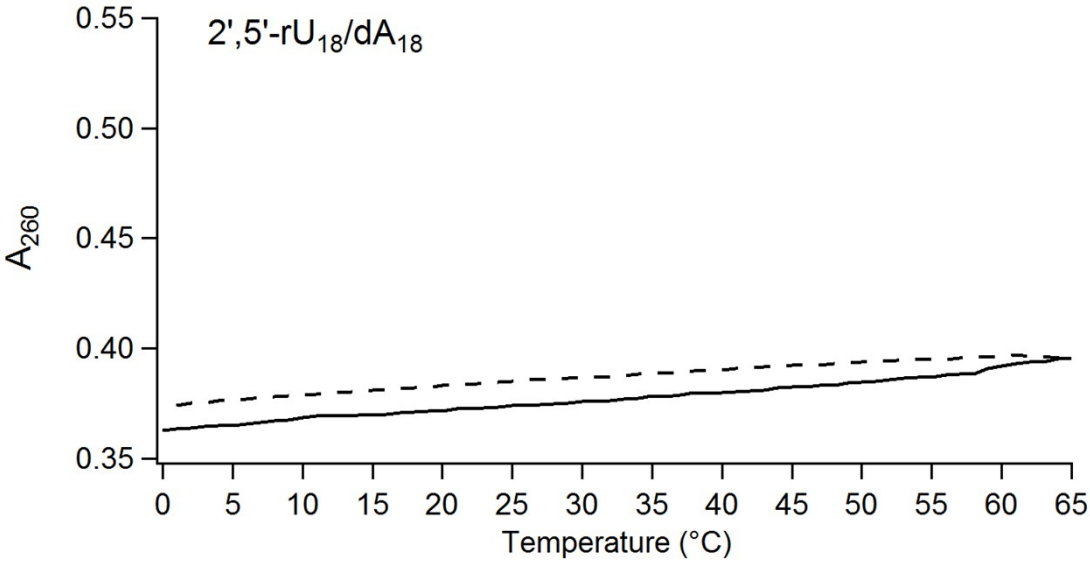

Fig. S1 UV-monitored thermal profiles of a solution containing 2',5'-rU_{18} and dA_{18} (5 μM in each oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dotted line. The solution contained 0.1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

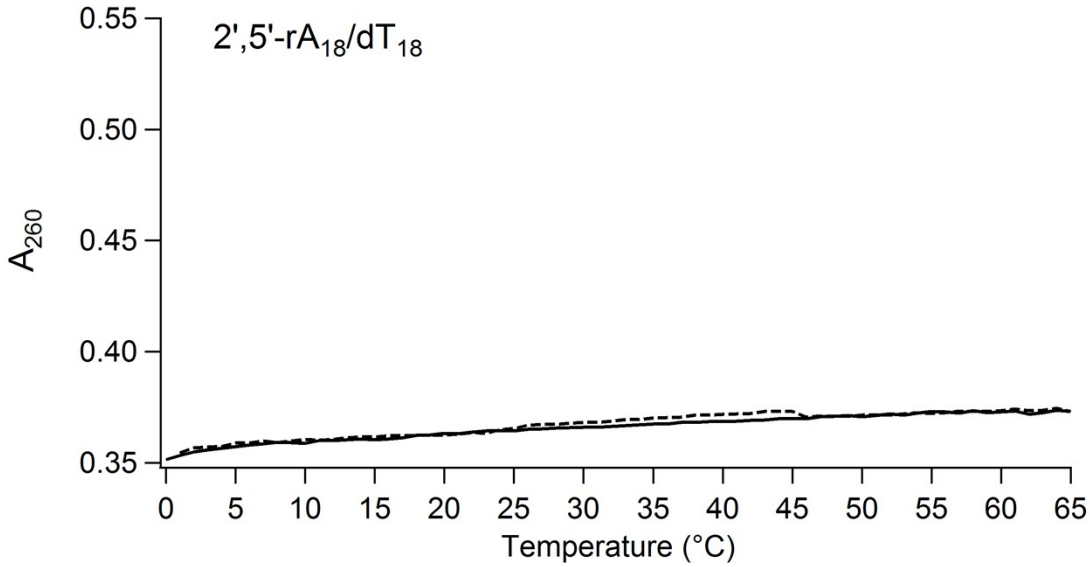

Fig. S2 UV-monitored thermal profiles of a solution containing 2',5'-rA_{18} and dT_{18} (5 μM in each oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dotted line. Solution contained 0.1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

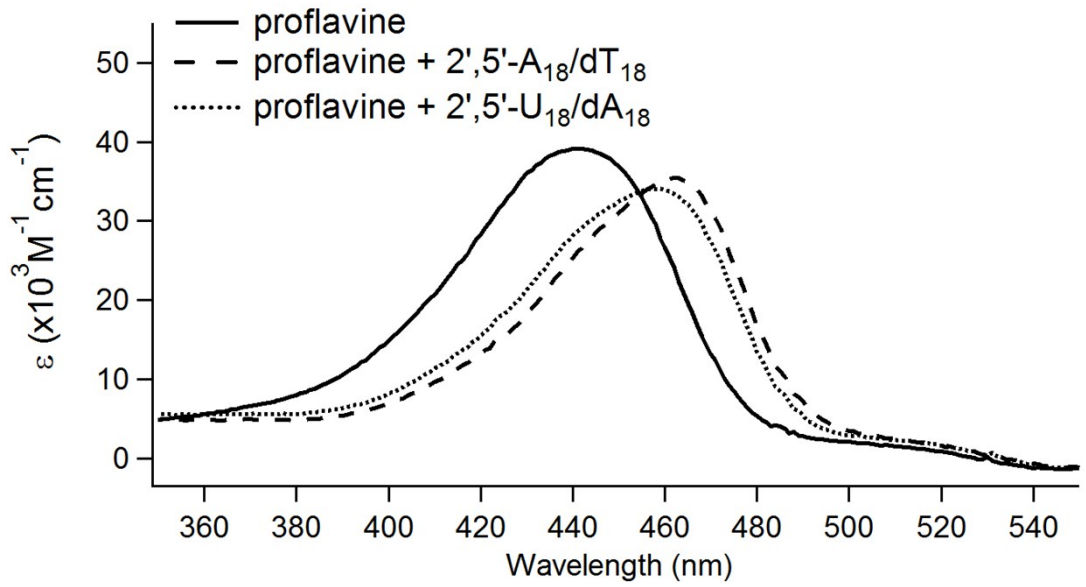

Fig. S3 UV spectra of proflavine in the absence (solid line) and in the presence of 2',5'-rU_{18} and dA_{18} (dotted line), or 2',5'-rA_{18} and dT_{18} (dashed line) at 4°C. Each solution was 45 μM in proflavine and 5 μM in each oligonucleotide (90 μM in base pair) for the solution containing 2',5'-rU_{18} and dA_{18}. Solutions contained 0.1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

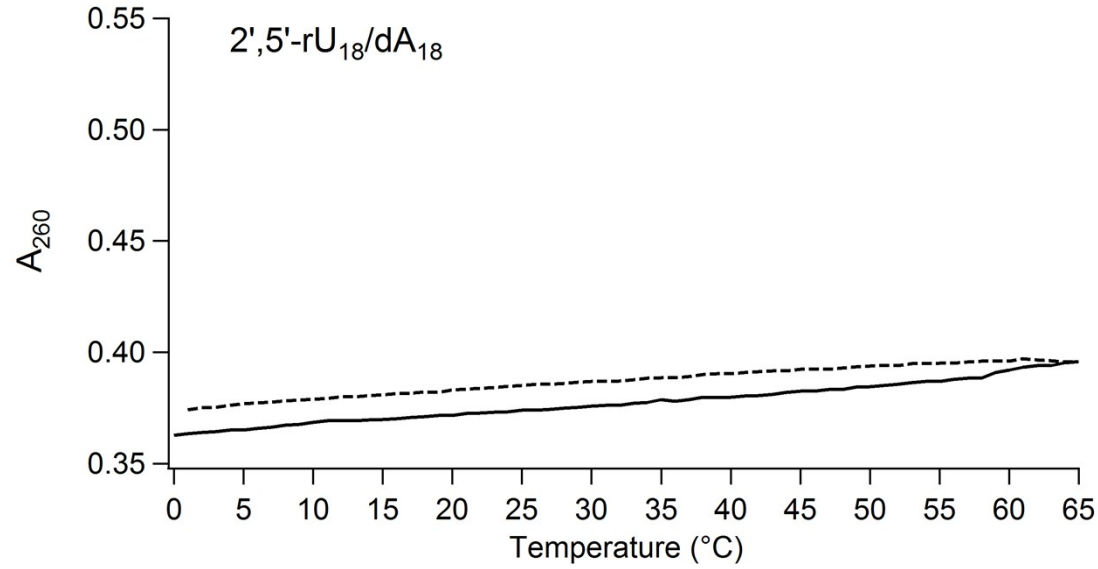

Fig. S4 UV-monitored thermal profiles of a solution containing 2',5'-rU_{18} and dA_{18} (5 μM in each oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. The solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

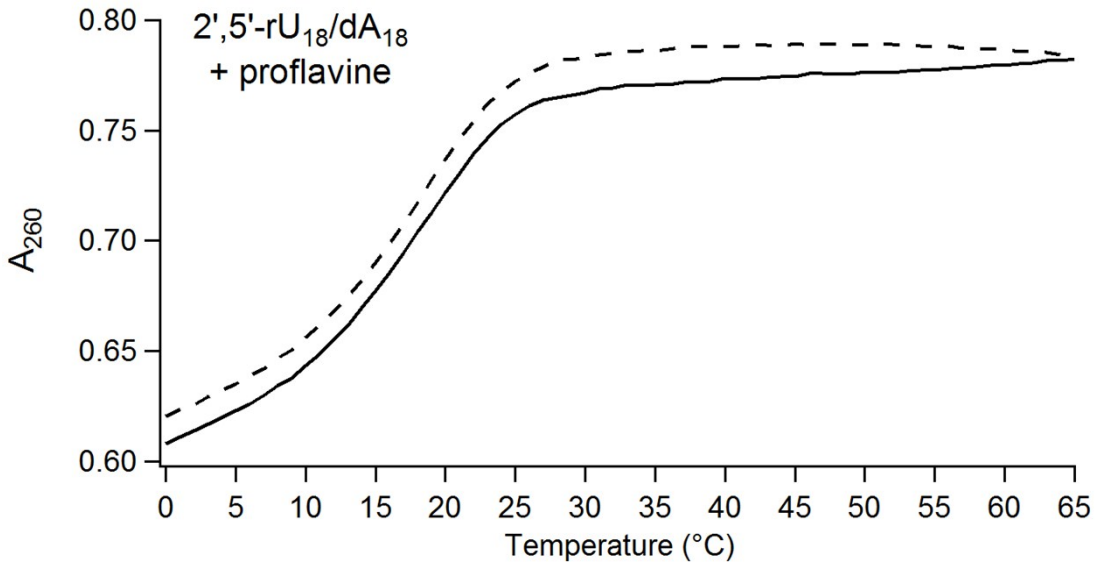

Fig. S5 UV-monitored thermal profiles of a solution containing 2',5'-rU_{18} and dA_{18} (5 μM in each oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

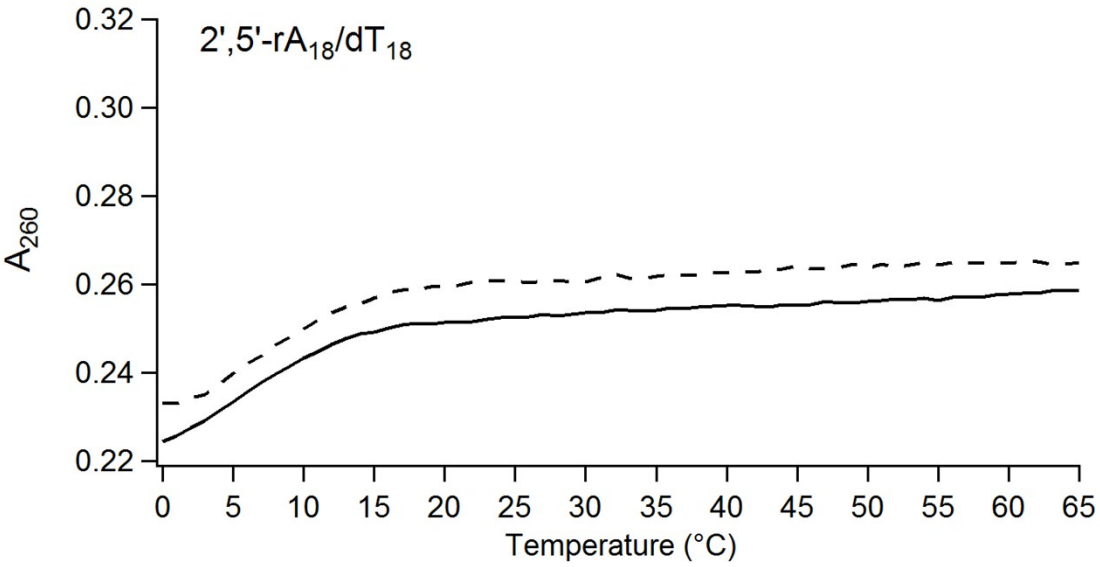

Fig. S6 UV-monitored thermal profiles of a solution containing 2',5'-rA_{18} and dT_{18} (5 μM in each oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0. Melt was performed in a 1 mm path length cell.

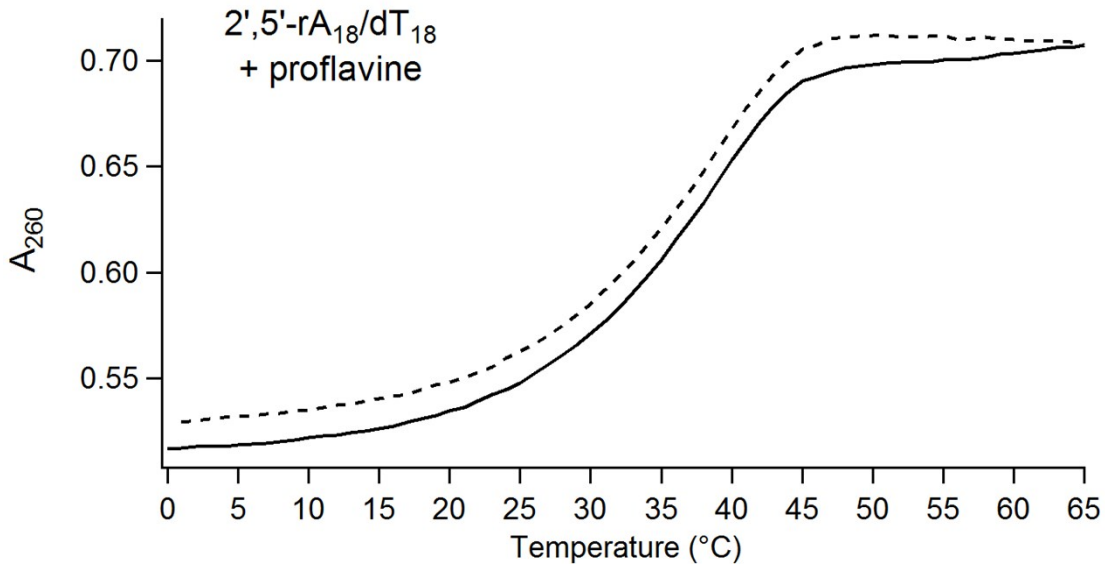

Fig. S7 UV-monitored thermal profiles of a solution containing 2',5'-rA_{18} and dT_{18} (5 μM in each oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

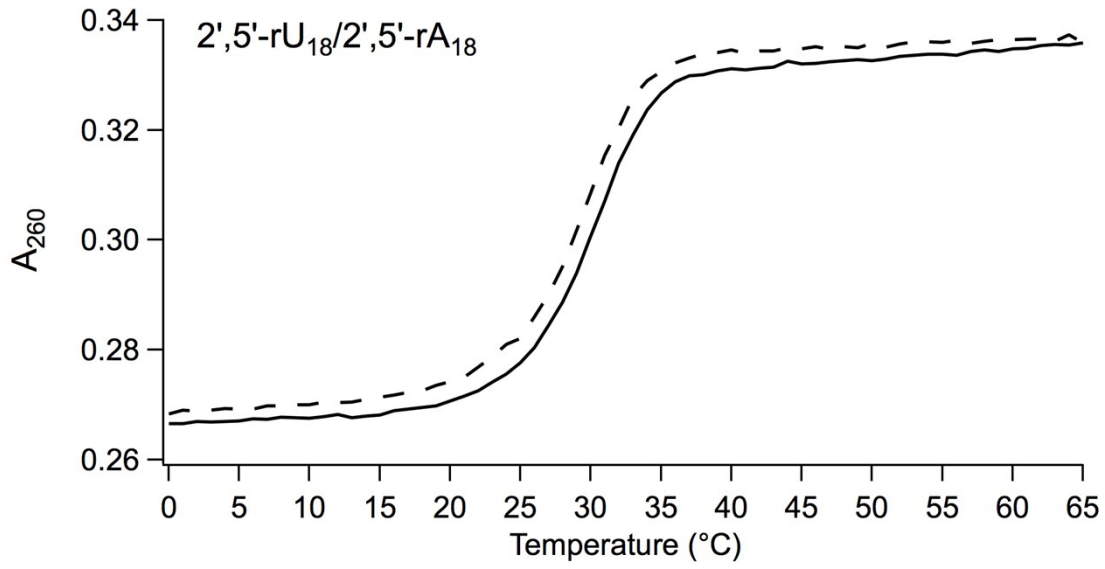

Fig. S8 UV-monitored thermal profiles of a solution containing 2',5'-rU_{18} and 2',5'-rA_{18} (5 μM in each oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

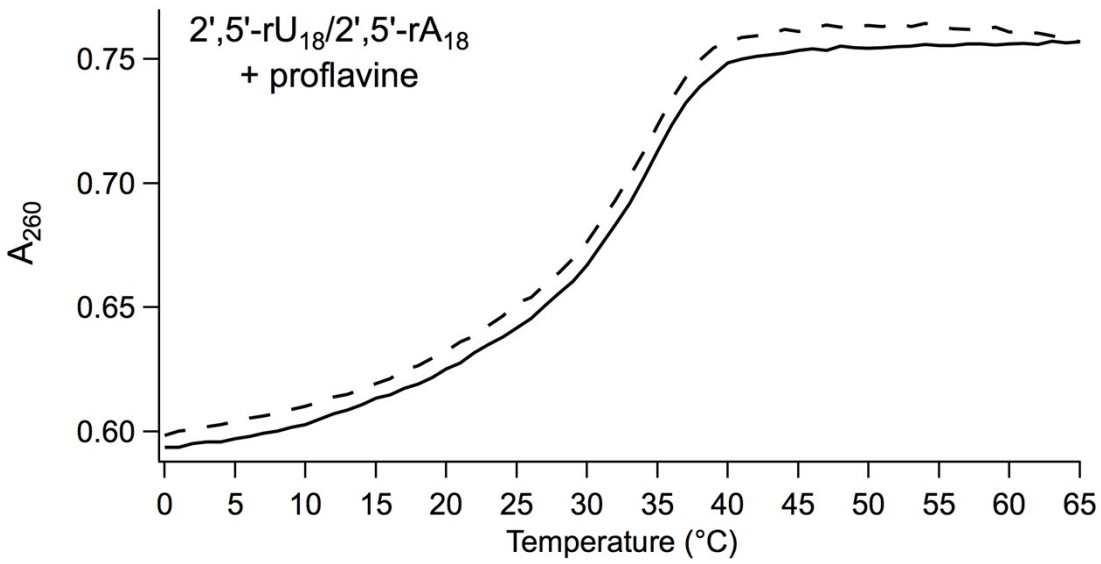

Fig. S9 UV-monitored thermal profiles of a solution containing 2',5'-rU_{18}, 2',5'-rA_{18} (5 μM in each oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

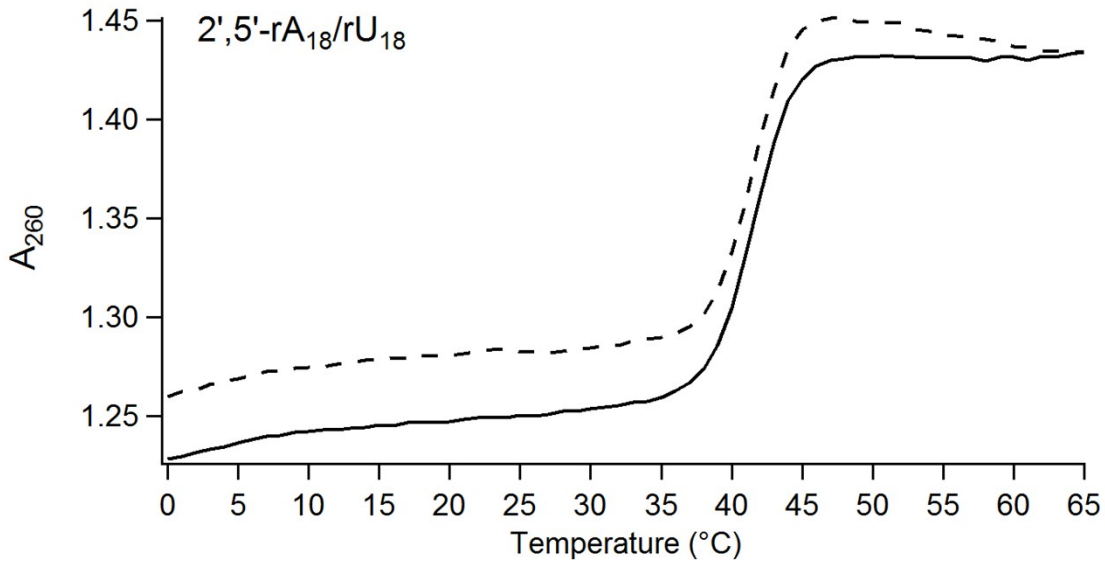

Fig. S10 UV-monitored thermal profiles of a solution containing 2',5'-rA_{18} and rU_{18} (5 μM in each oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

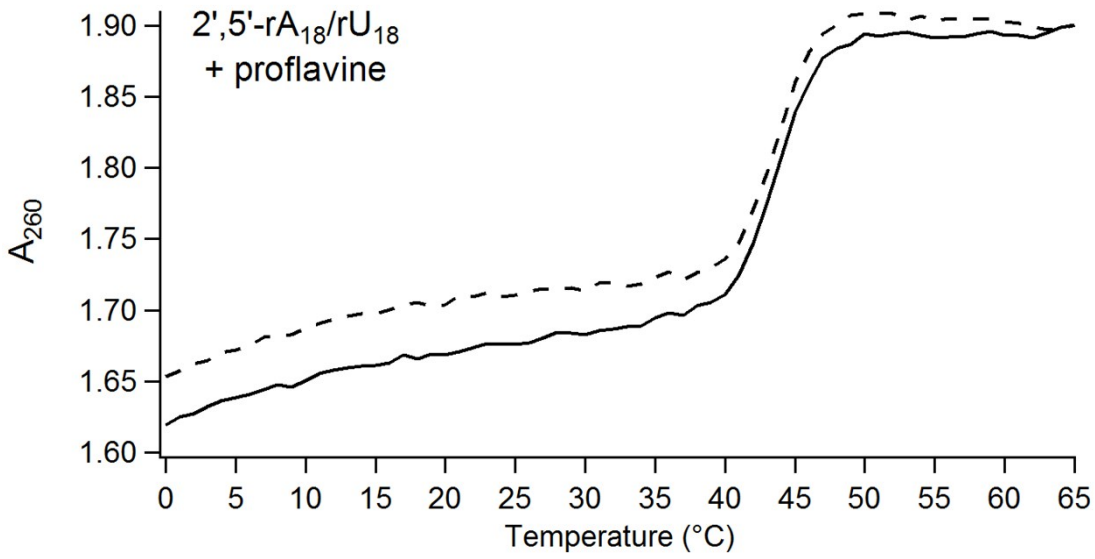

Fig. S11 UV-monitored thermal profiles of a solution containing 2',5'-rA_{18} and rU_{18} (5 μM in each oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

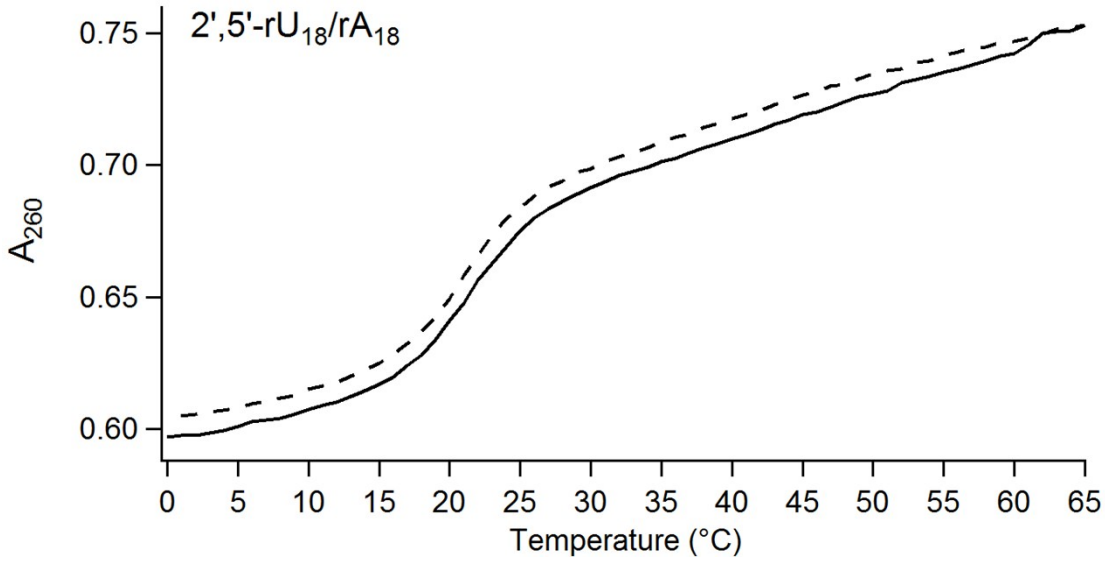

Fig. S12 UV-monitored thermal profiles of a solution containing 2',5'-rU_{18} and rA_{18} (5 μM in each oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

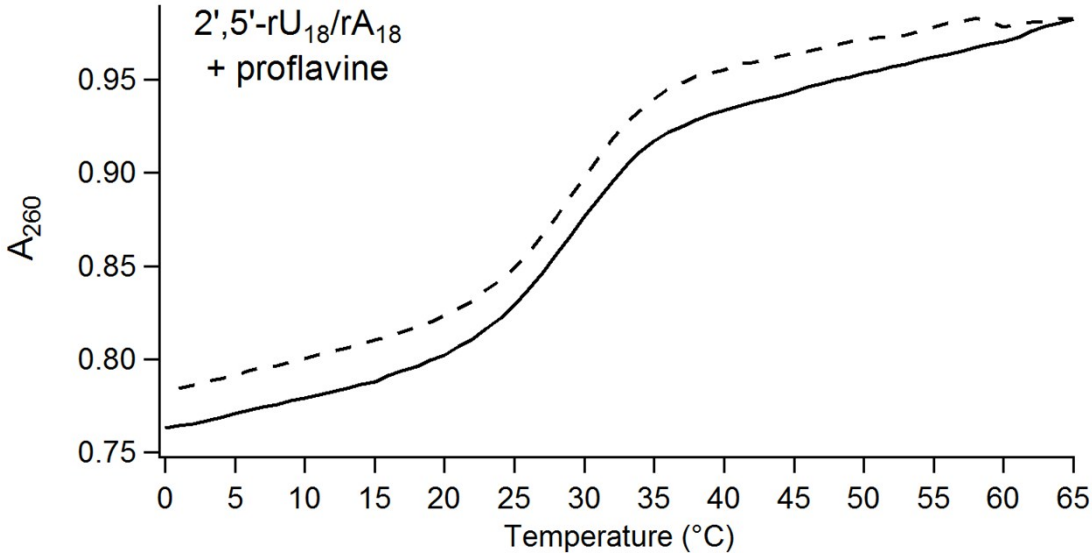

Fig. S13 UV-monitored thermal profiles of a solution containing 2',5'-rU_{18} and rA_{18} (5 μM in each oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

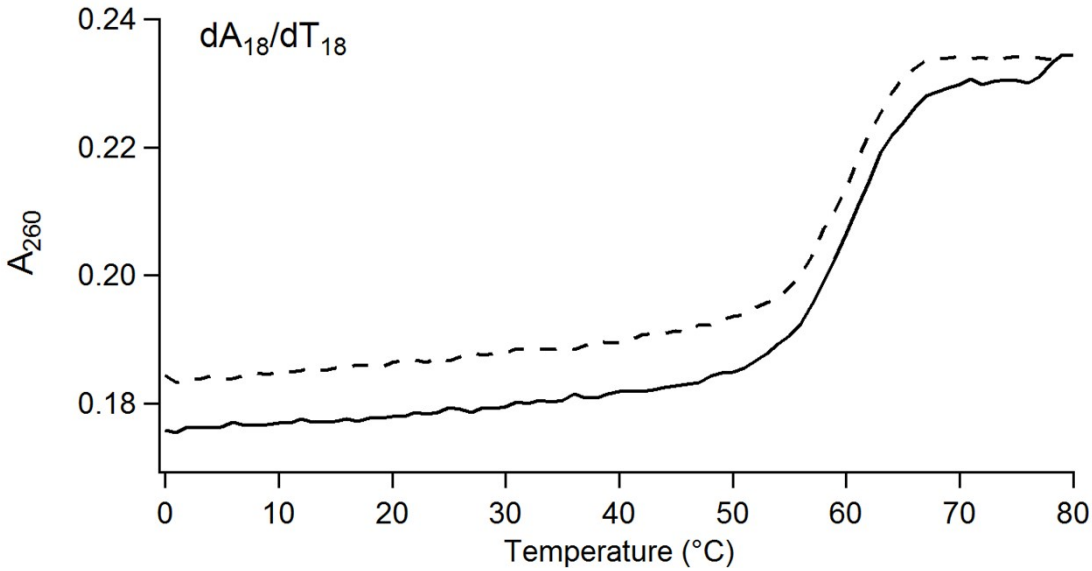

Fig. S14 UV-monitored thermal profiles of a solution containing dA_{18} and dT_{18} (5 μM in each oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0. Melt was performed in a 1 mm path length cell.

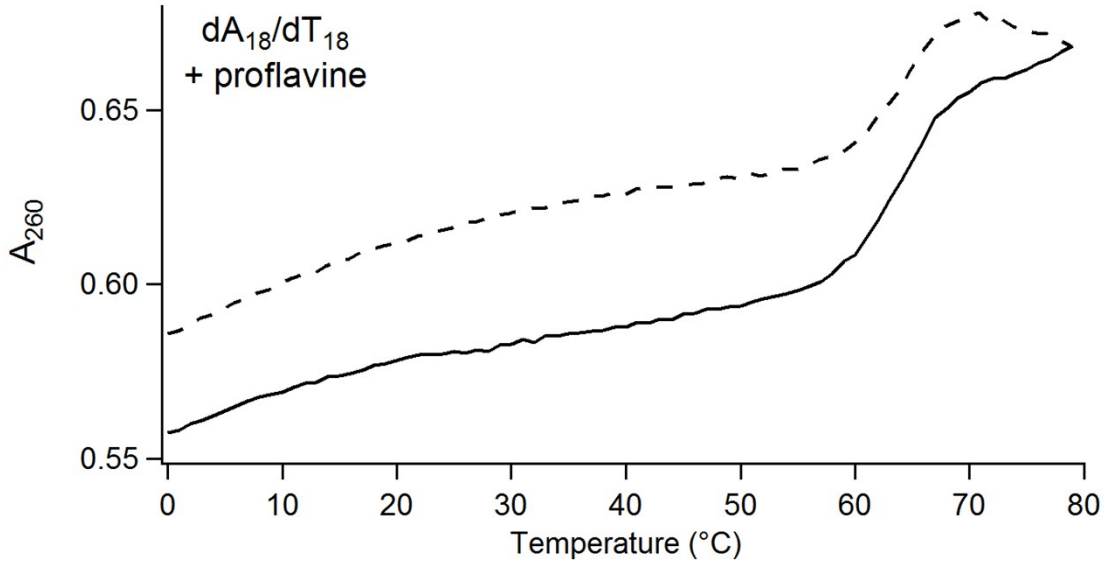

Fig. S15 UV-monitored thermal profiles of a solution containing dA_{18} and dT_{18} (5 μM in each oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dahsed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0. Melt was performed in a 1 mm path length cell.

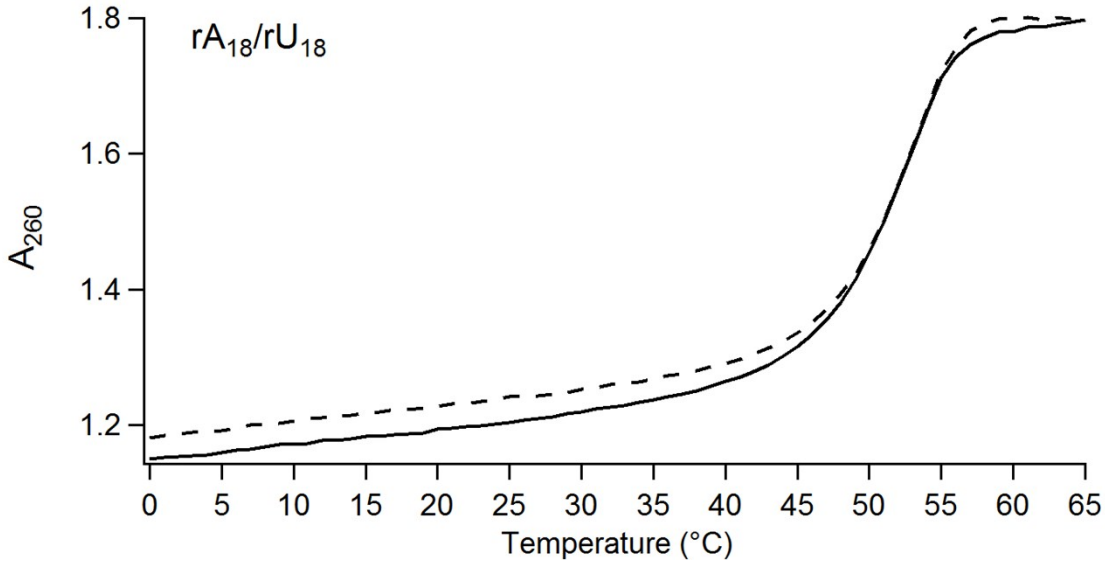

Fig. S16 UV-monitored thermal profiles of a solution containing rA_{18} and rU_{18} (5 μM in each oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

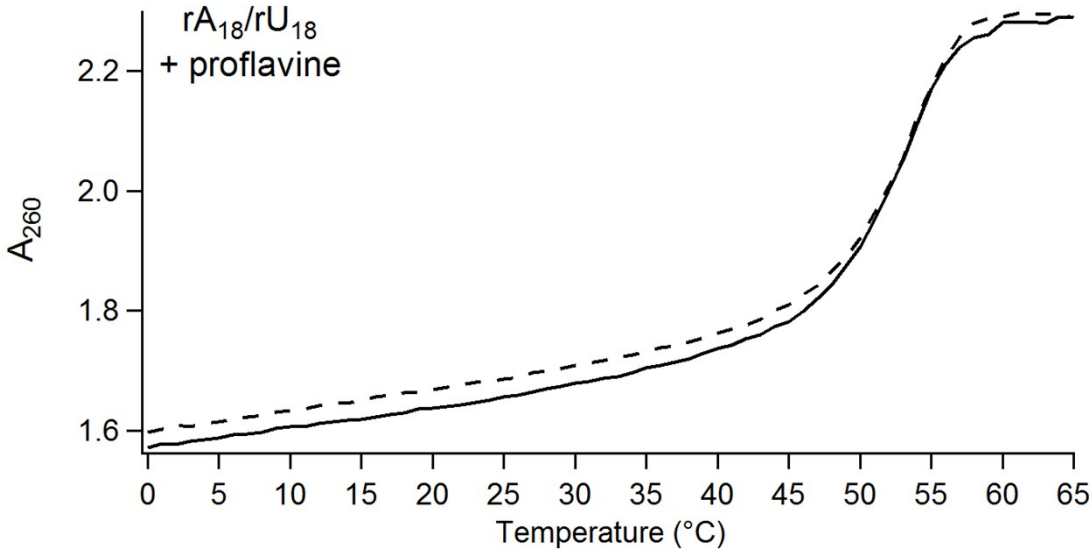

Fig. S17 UV-monitored thermal profiles of a solution containing rA_{18} and rU_{18} (5 μM in each oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

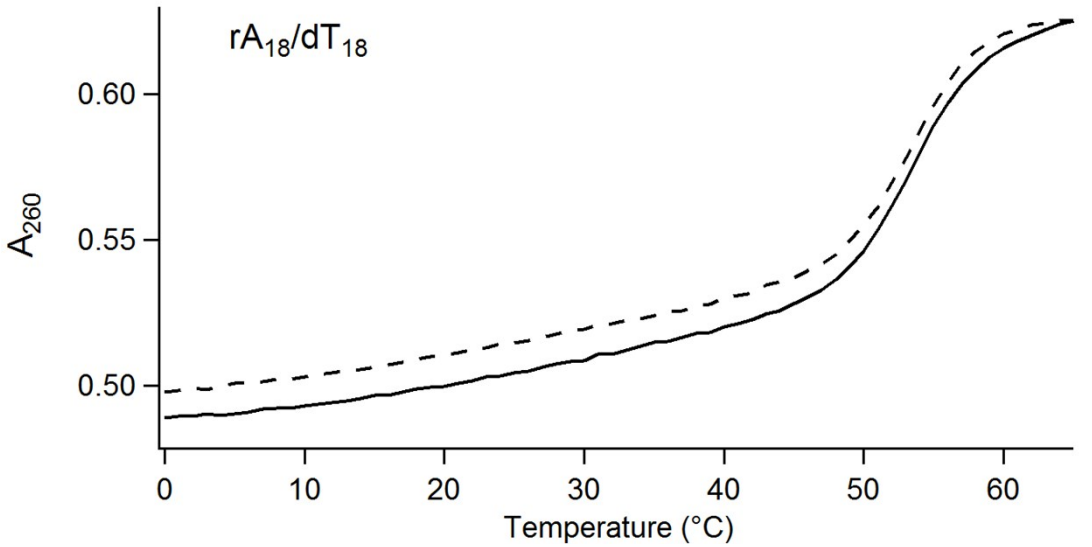

Fig. S18 UV-monitored thermal profiles of a solution containing rA_{18} and dT_{18} (5 μM in each oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

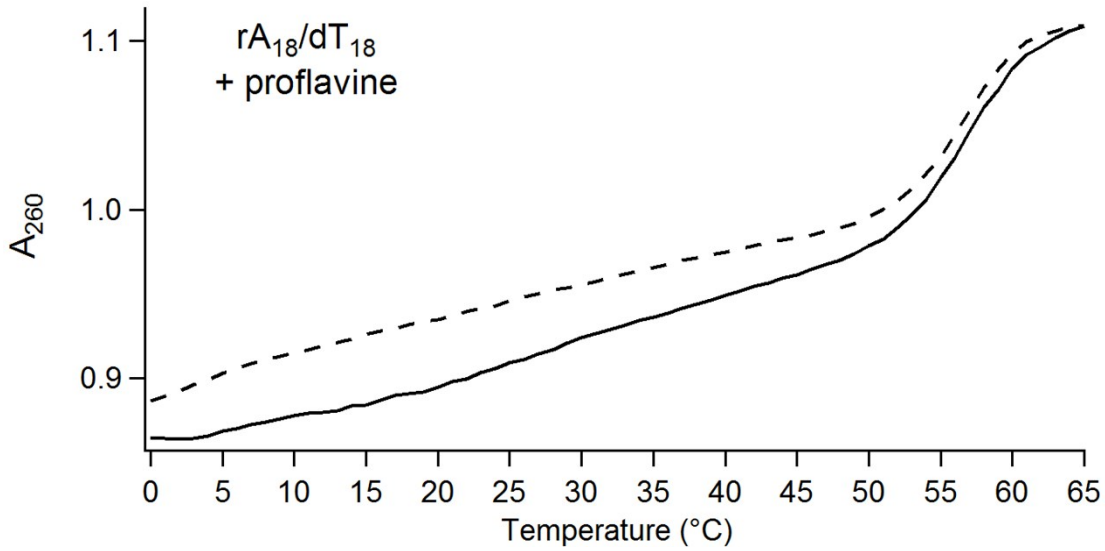

Fig. S19 UV-monitored thermal profiles of a solution containing rA_{18} and dT_{18} (5 μM in each oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

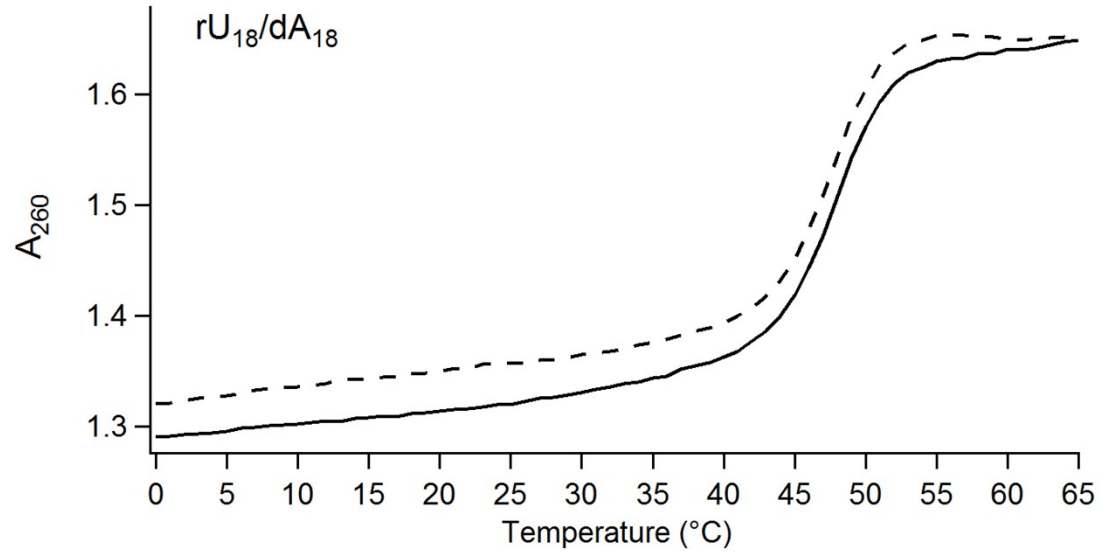

Fig. S20 UV-monitored thermal profiles of a solution containing rU_{18} and dA_{18} (5 μM in each oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

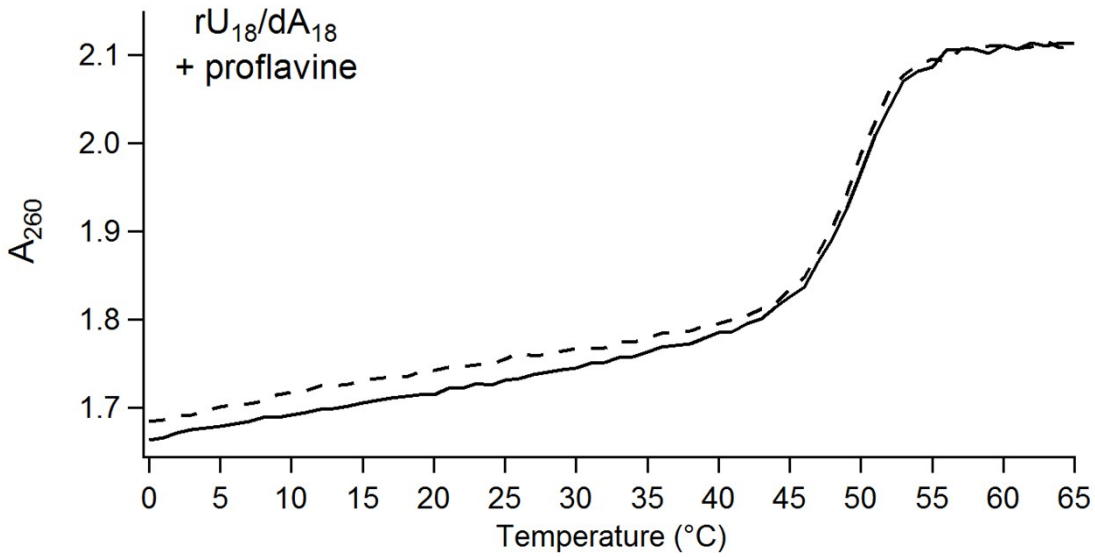

Fig. S21 UV-monitored thermal profiles of a solution containing rU_{18} and dA_{18} (5 μM in each oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

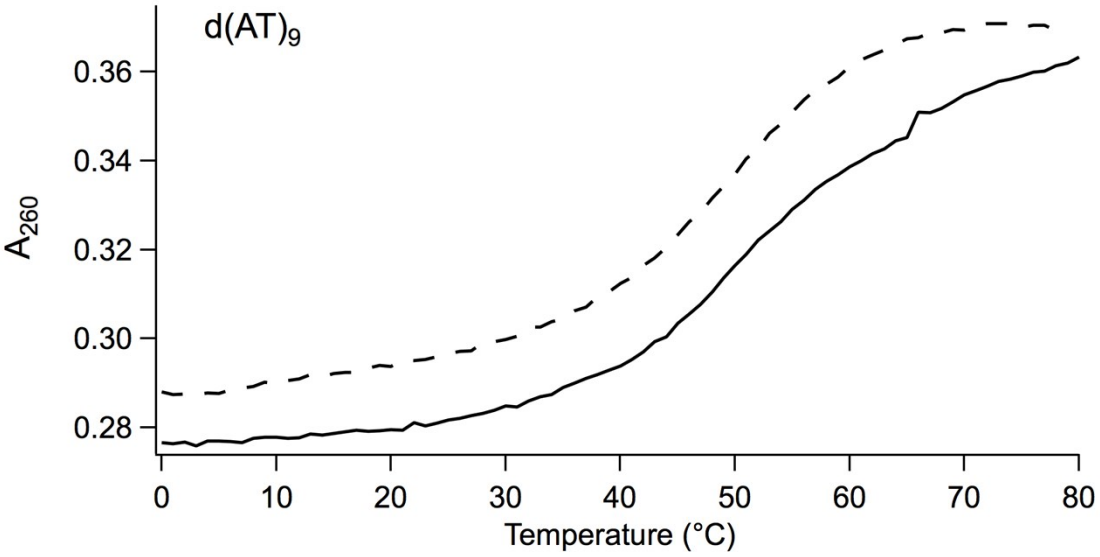

Fig. S22 UV-monitored thermal profiles of a solution containing $d(AT)_9$ (10 μM in oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0. Melt was performed in a 1 mm path length cell.

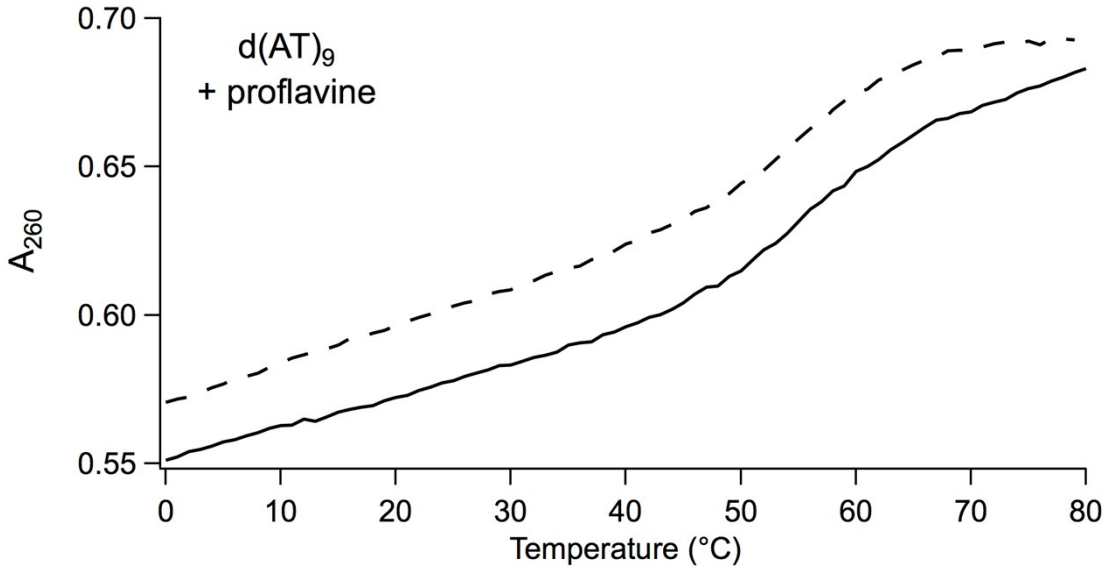

Fig. S23 UV-monitored thermal profiles of a solution containing $d(AT)_9$ (10 μM in oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0. Melt was performed in a 1 mm path length cell.

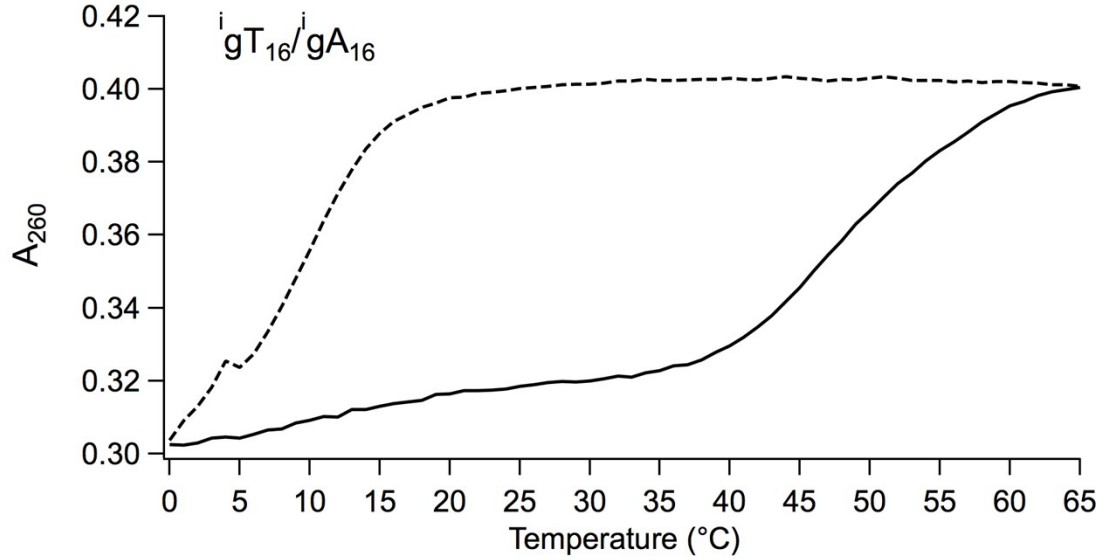

Fig. S24 UV-monitored thermal profiles of a solution containing $^{i}gT_{16}$ and $^{i}gA_{16}$ (5 μM in each oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

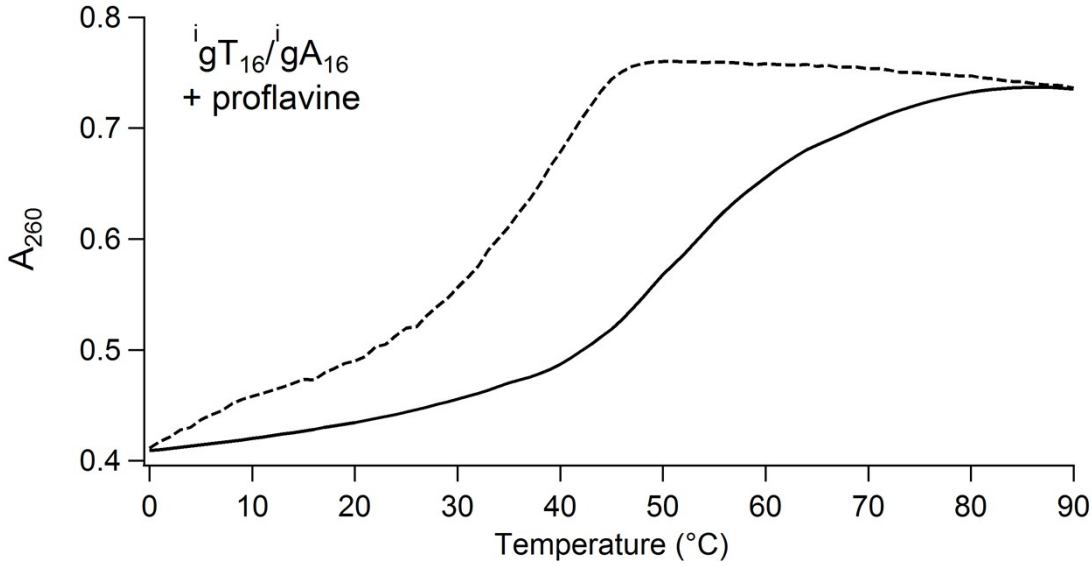

Fig. S25 UV-monitored thermal profiles of a solution containing $^{i}gT_{16}$, $^{i}gA_{16}$ (5 μM in each oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

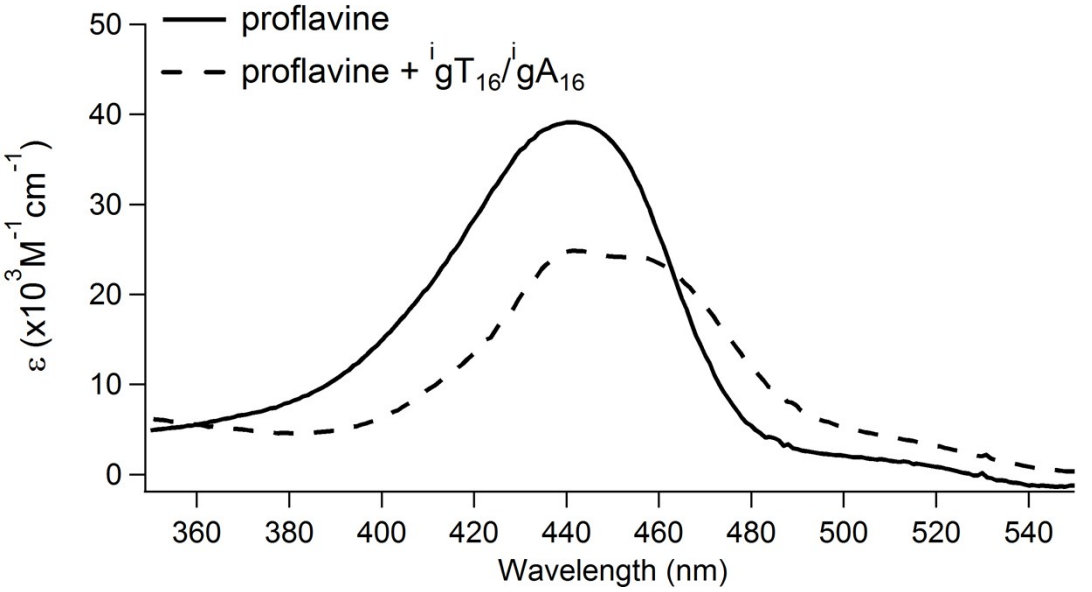

Fig. S26 UV spectra of proflavine in the absence (solid line) and in the presence of $^{i}gT_{16}$ and $^{i}gA_{16}$ (dashed line) at 4 °C. Each solution was 45 μM in proflavine and 5 μM in each oligonucleotide (90 μM in base pair) for the solution containing 2',5'-rU_{18} and dA_{18}. Solutions contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

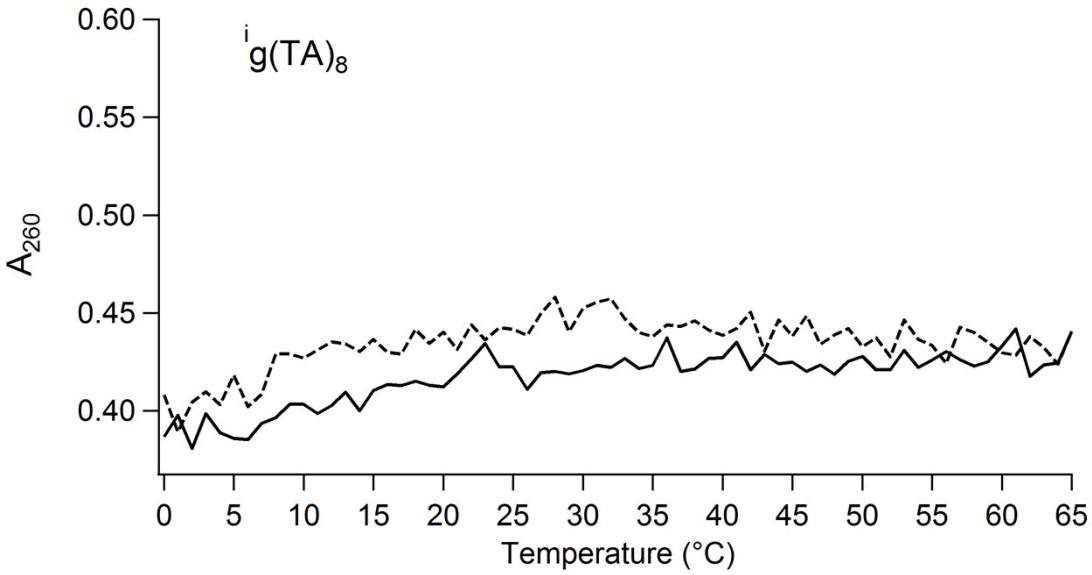

Fig. S27 UV-monitored thermal profiles of a solution containing $^{i}g(TA)_8$ (10 μM in oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. The solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

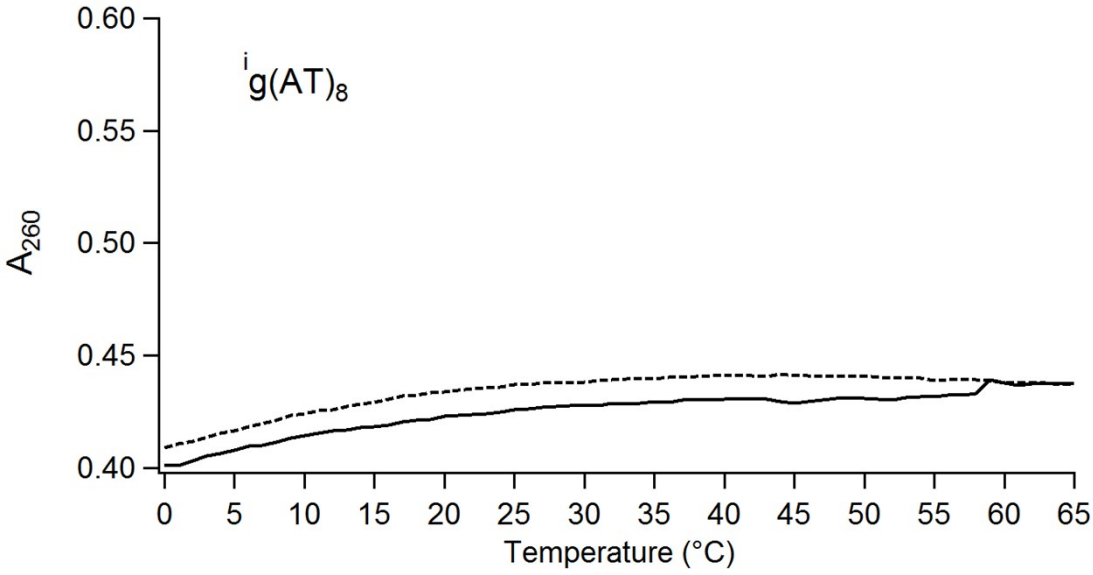

Fig. S28 UV-monitored thermal profiles of a solution containing $^{i}g(AT)_8$ (10 μM in oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. The solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

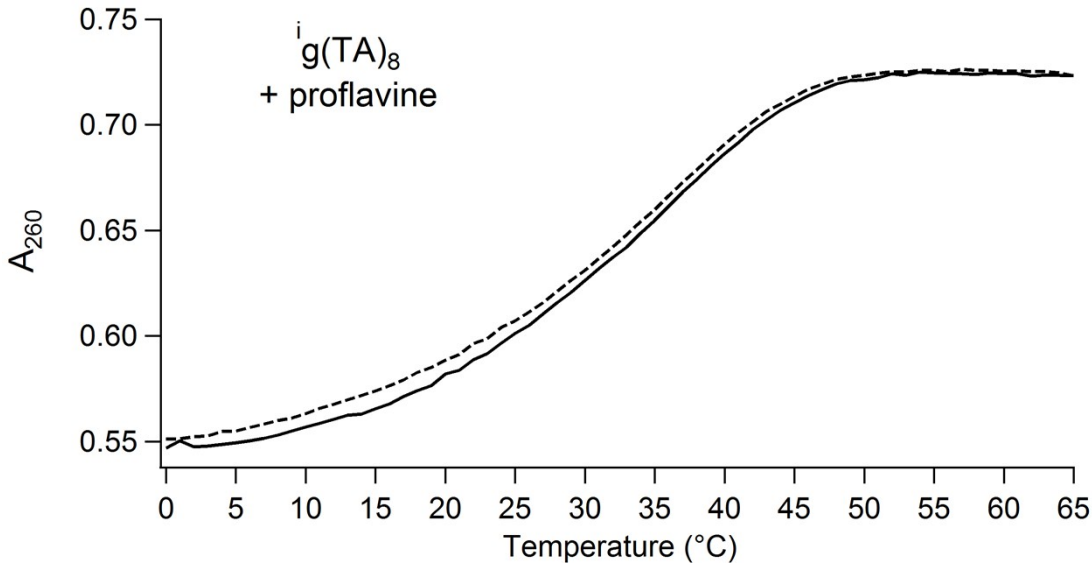

Fig. S29 UV-monitored thermal profiles of a solution containing ^{i}g(TA)$_8$ (10 μM in oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dahsed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

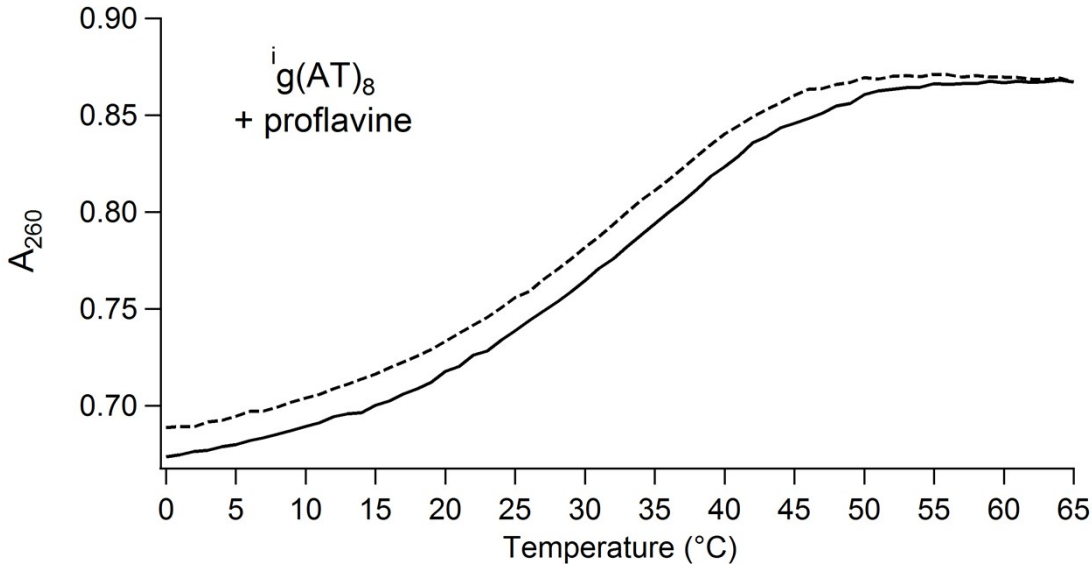

Fig. S30 UV-monitored thermal profiles of a solution containing ^{i}g(AT)$_8$ (10 μM in oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

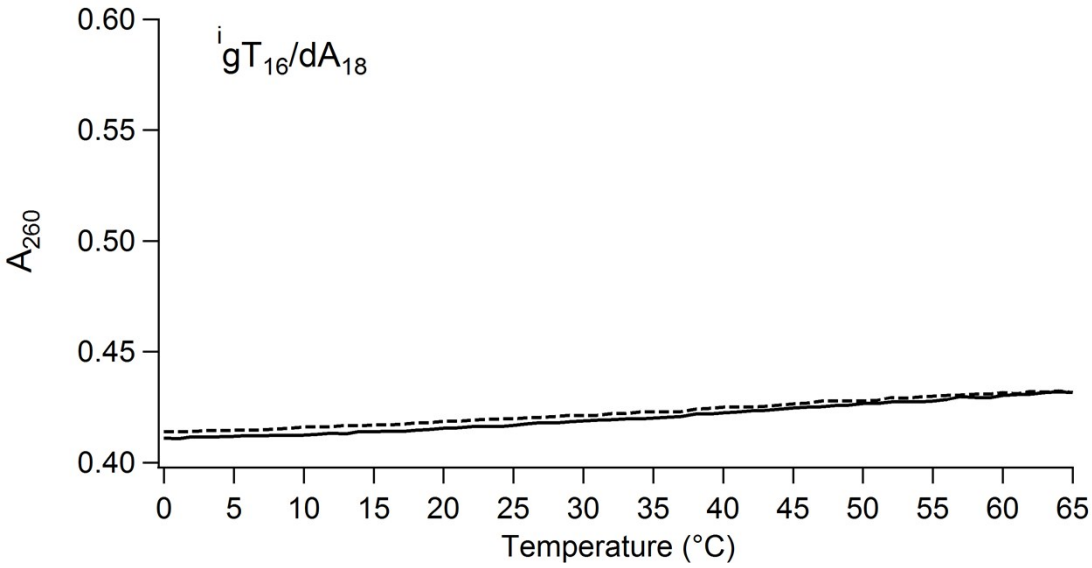

Fig. S31 UV-monitored thermal profiles of a solution containing $^{i}gT_{16}$ and dA_{18} (5 μM in each oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. The solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

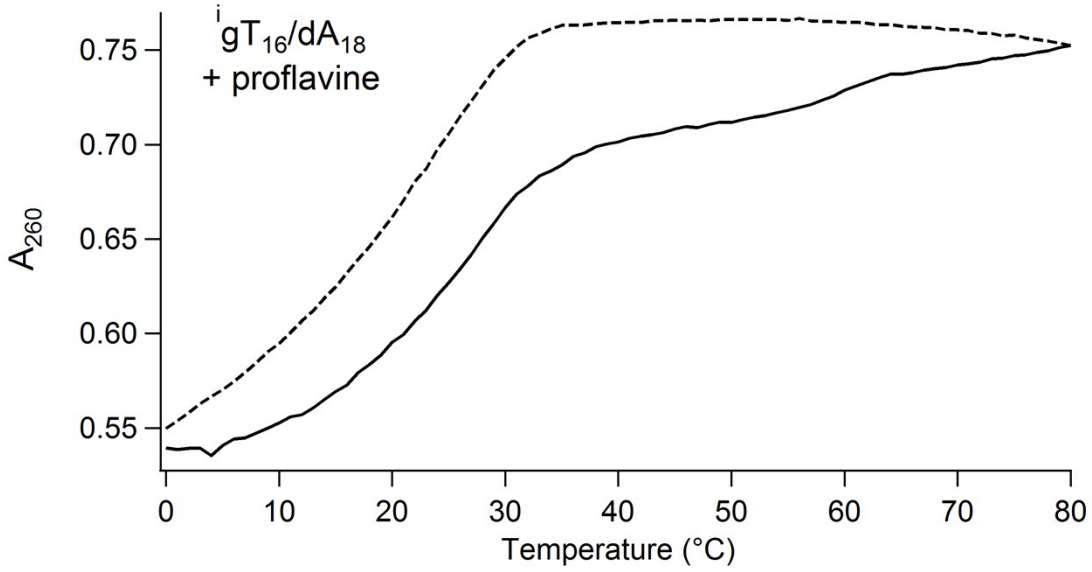

Fig. S32UV-monitored thermal profiles of a solution containing $^{i}gT_{16}$, dA_{18} (5 μM each in oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

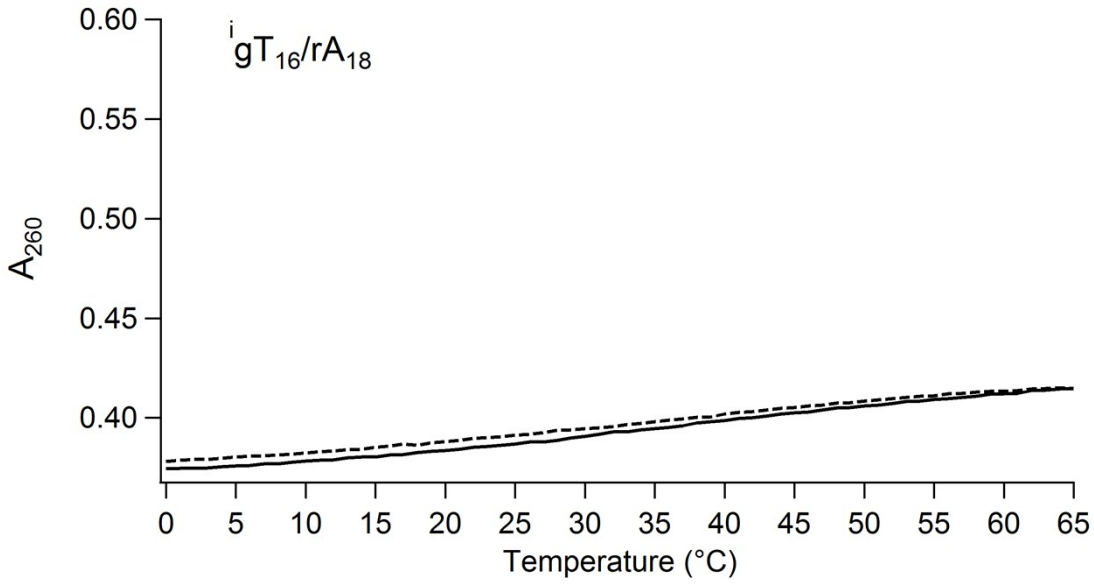

Fig. S33 UV-monitored thermal profiles of a solution containing $^{i}gT_{16}$ and rA_{18} (5 μM in each oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. The solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

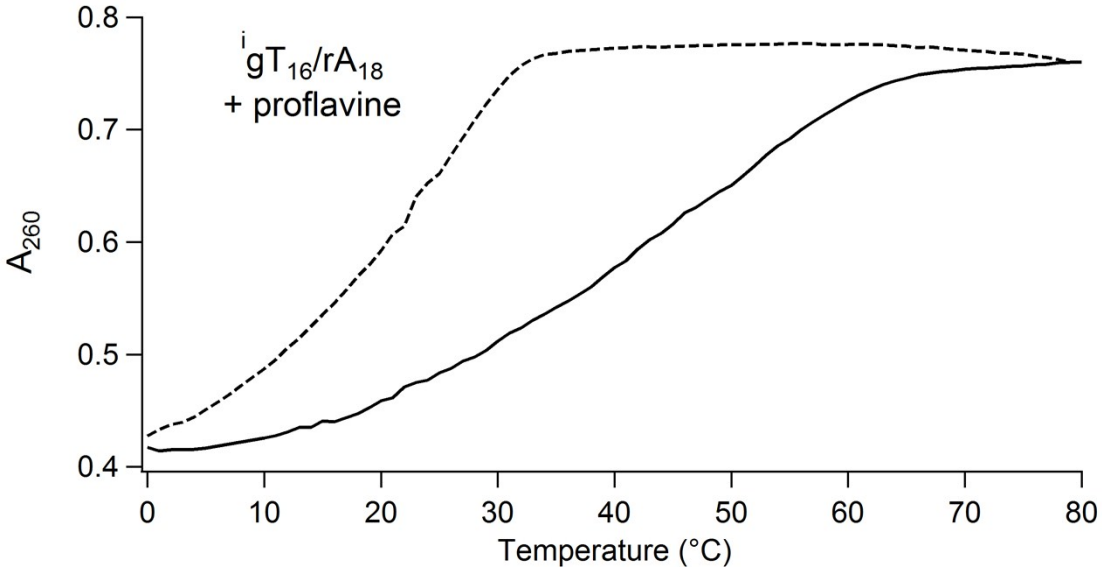

Fig. S34 UV-monitored thermal profiles of a solution containing $^{i}gT_{16}$, rA_{18} (5 μM in each oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

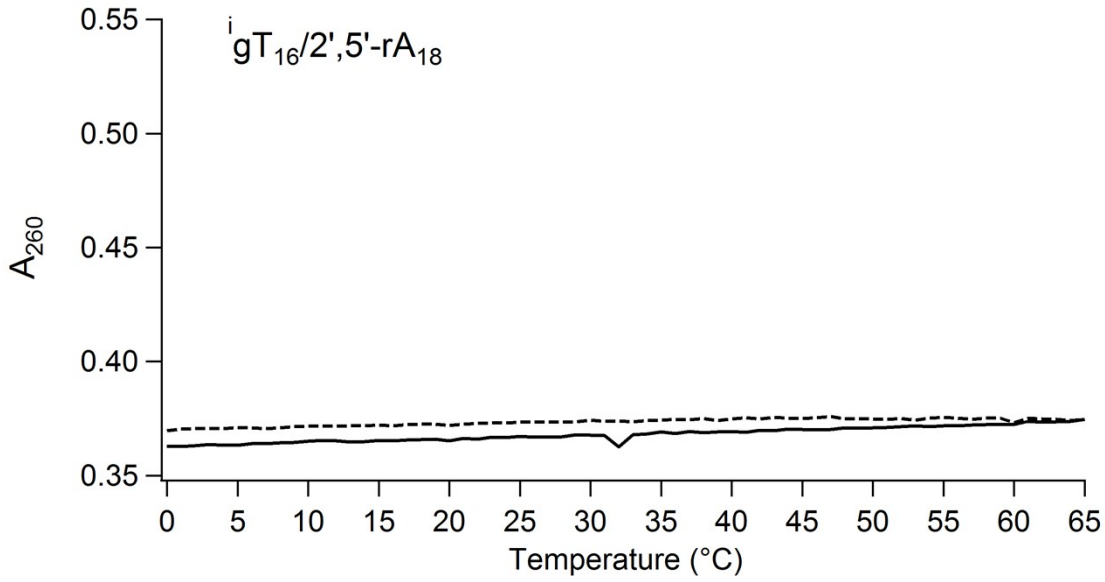

Fig. S35 UV-monitored thermal profiles of a solution containing ^{i}gT$_{16}$ and 2',5'-dA$_{18}$ (5 μM in each oligonucleotide). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. The solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

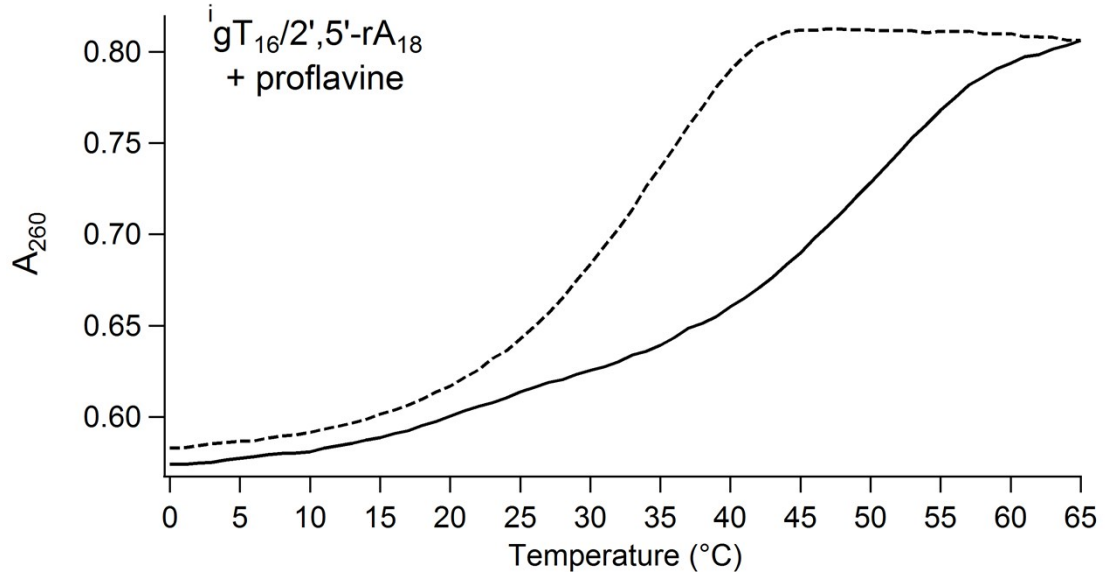

Fig. S36 UV-monitored thermal profiles of a solution containing ^{i}g(T)$_{16}$, 2',5'-rA$_{18}$ (5 μM in each oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0. This particular duplex appears to have a pre-melting transition around 20 °C.

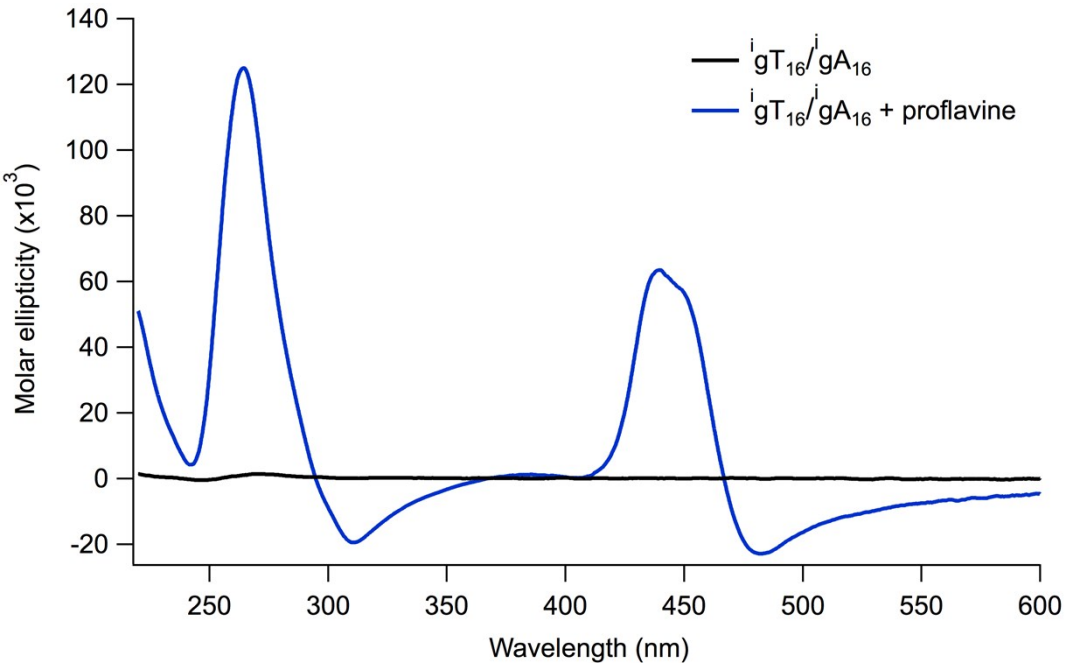

Fig. S37 CD spectra of the $^{i}gT_{16}/^{i}gA_{16}$ duplex in the adsence (black line) and in the presence (blue line) of proflavine. Both solutions were 10 μM in each oligonucleotide. Sample containing proflavine was 90 μM in proflavine. Solutions contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

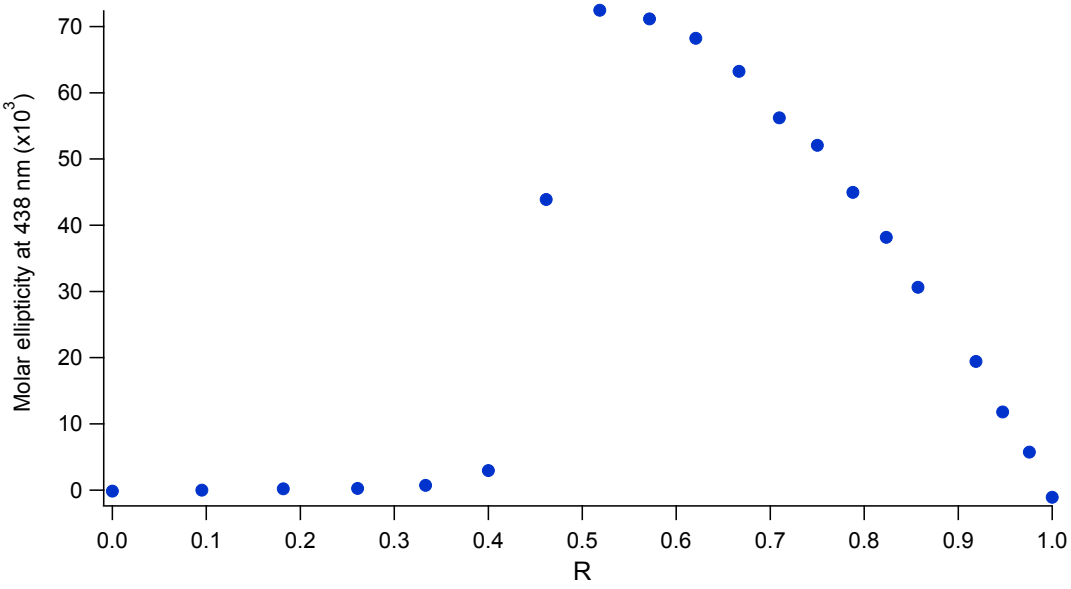

Fig. S38 Job plot analysis of the binding of proflavine to the $^{i}gT_{16}/^{i}gA_{16}$ duplex. R = [proflavine]/([proflavine]+[base pair]/2). At R = 0.5, concentration of each oligonucleotide is 5 μM (90 μM in base pair) and proflavine is 45 μM. All solutions contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0. Measurements were performed at 4 °C.

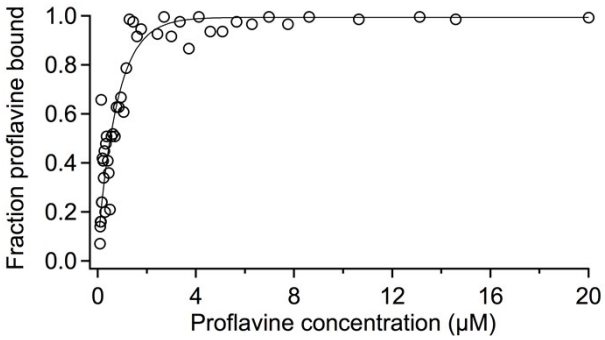

Fig S39 Data used for determination of the association constant (K_a) of proflavine with the duplex $^{i}gT_{16}/^{i}gA_{16}$ at 4 °C. Serial dilutions were performed using a solution originally 5 μM in each oligonucleotide and 45 μM in proflavine (one proflavine per two base pairs). The change in proflavine absorbance upon dissociation from the isoGNA duplex was used to monitor the fraction of proflavine bound to the duplex (normalized between 0 and 1). The K_a was determined to be 2 x 10^6 M^{-1}. All solutions contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

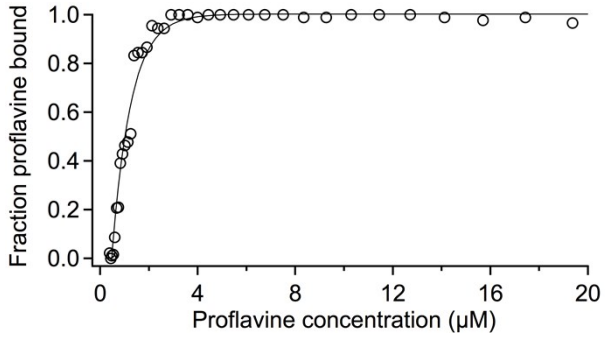

Fig. S40 Data used for determination of the association constant (K_a) of proflavine with the duplex $^{i}g(AT)_8$ at 4 °C. Serial dilutions were performed using a solution originally 10 μM in oligonucleotide and 45 μM in proflavine (one proflavine per two base pairs). The change in proflavine UV absorbance upon dissociation from the isoGNA duplex was used to monitor the fraction of proflavine bound to the duplex (normalized here between 0 and 1). The K_a was determined to be 1 x 10^6 M^{-1}. All solutions contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

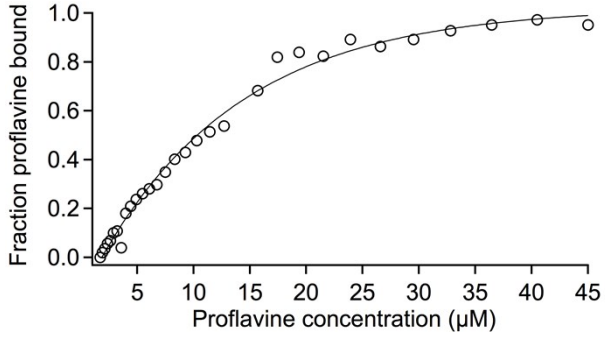

Fig. S41 Data used for determination of the association constant (K_a) of proflavine with the duplex $d(AT)_9$ at 4 °C. Serial dilutions were performed using a solution originally 10 μM in oligonucleotide and 45 μM in proflavine (one proflavine per two base pairs). The change in proflavine UV absorbance upon dissociation from the DNA duplex was used to monitor the fraction of proflavine bound to the duplex (normalized here between 0 and 1). The K_a was determined to be 8.3 x 10^4 M^{-1}. All solutions contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

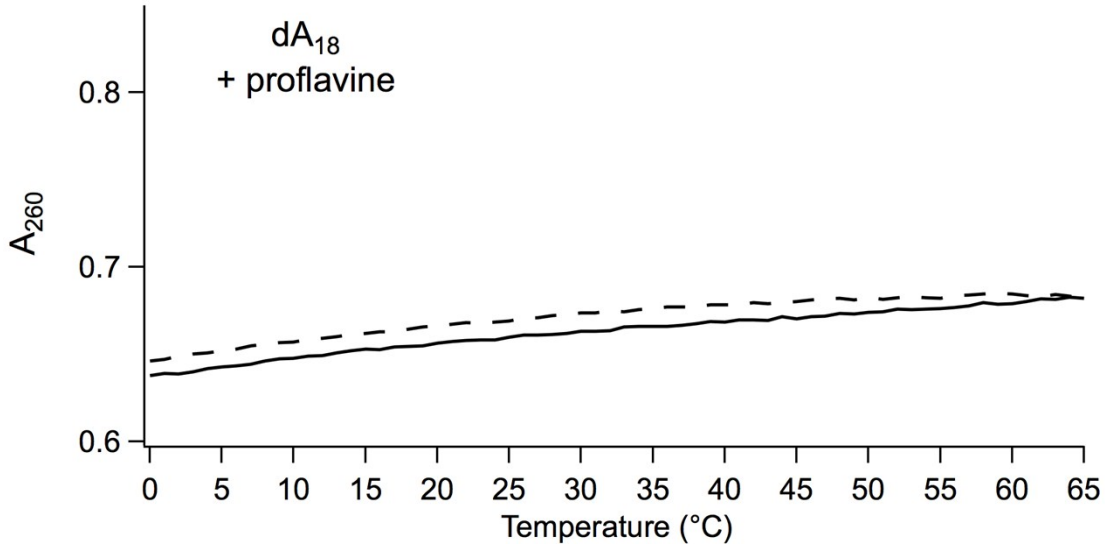

Fig. S42 UV-monitored thermal profiles of a solution containing dA_{18} (5 μM in oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

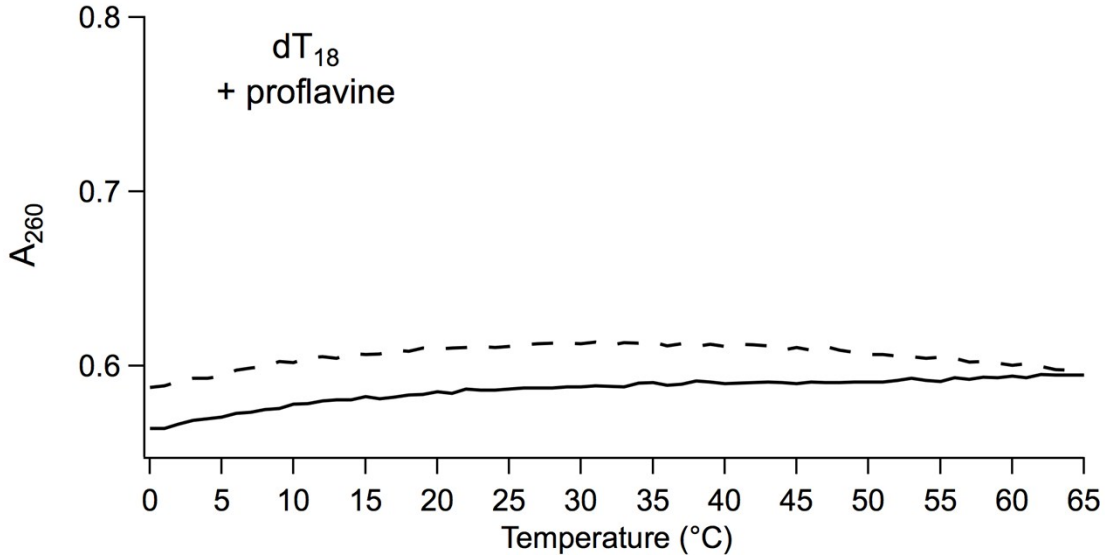

Fig. S43 UV-monitored thermal profiles of a solution containing dT_{18} (5 μM in oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

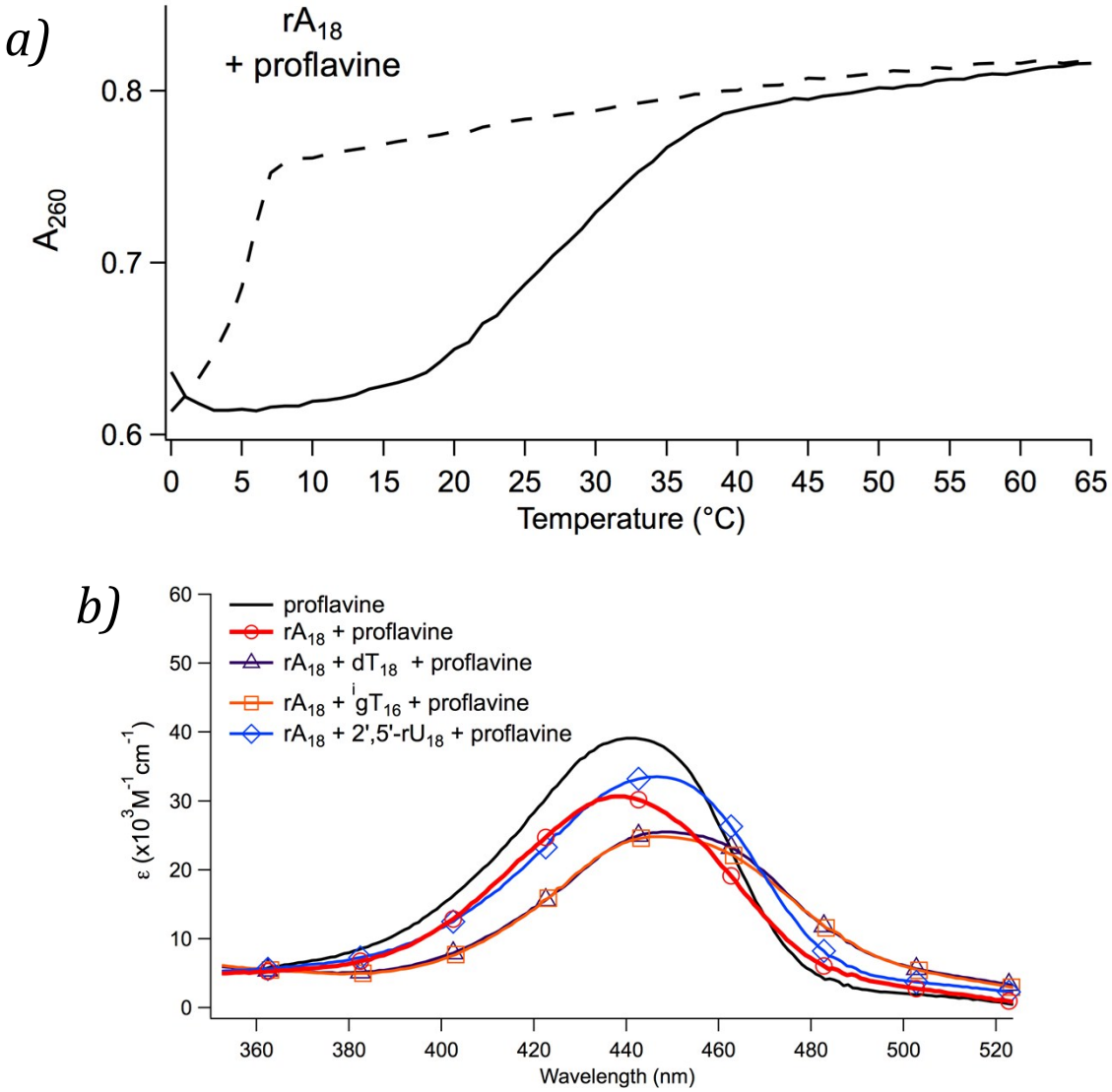

Fig. S44 *a)* UV-monitored thermal profiles of a solution containing rA_{18} (5 μM in oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. rA_{18} forms a self-structure with proflavine with T_m of 28 °C and 5 °C for heating and cooling, respectively. We note that the self-structure formed by rA_{18} in the presence of proflavine is less stable than the duplexes formed with rA_{18} as one strand in the presence if proflavine. *b)* UV/vis spectra of longest wavelength proflavine absorption bands for solutions containing poflavine in the absence of oligonucleotide (solid black line), proflavine in the presence of rA_{18} only (red line-open circles), proflavine in the presence of rA_{18} and dT_{18} (purple line-open trangles), proflavine in the presence of rA_{18} and $^{i}gT_{16}$ (orange line-open squares), and proflavine in the presence of rA_{18} and 2',5'-rU_{18} (blue line-open diamonds). Note that the maximum of the proflavine absorption band is shifted to lower wavelengths (blue shift) for solutions containing proflavine and only rA_{18} relative to a solution with proflavine in the absence of oligonucleotide. The maxima of the proflavine absorption band is shifted to higher wavelengths (red shift) for solutions containing proflavine, rA_{18} and a complementary nucleic acid (i.e., dT_{18}, igT_{16}, 2',5'-rU_{18}). A red shift in the absorption profile supports intercalative binding of proflavine to nucleic acid duplexes. Solutions contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

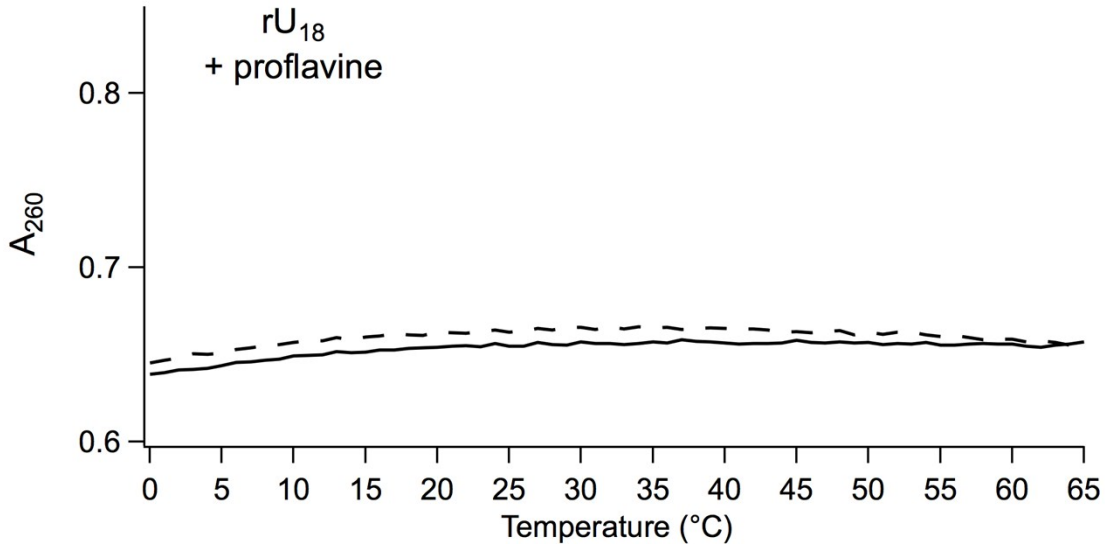

Fig. S45 UV-monitored thermal profiles of a solution containing rU_{18} (5 μM in oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

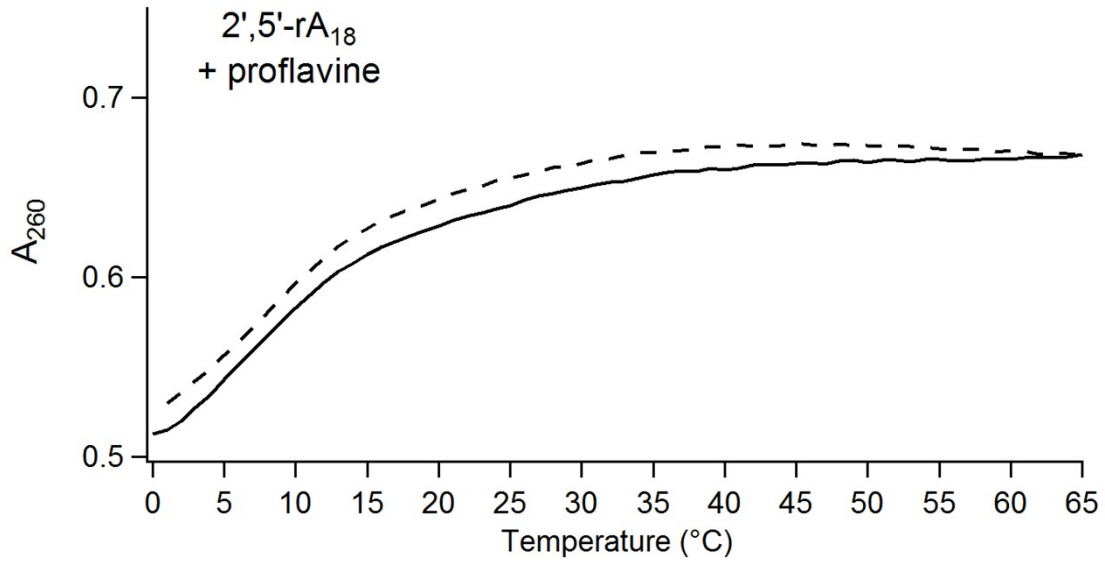

Fig. S46 UV-monitored thermal profiles of a solution containing 2',5'-rA_{18} (5 μM in oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

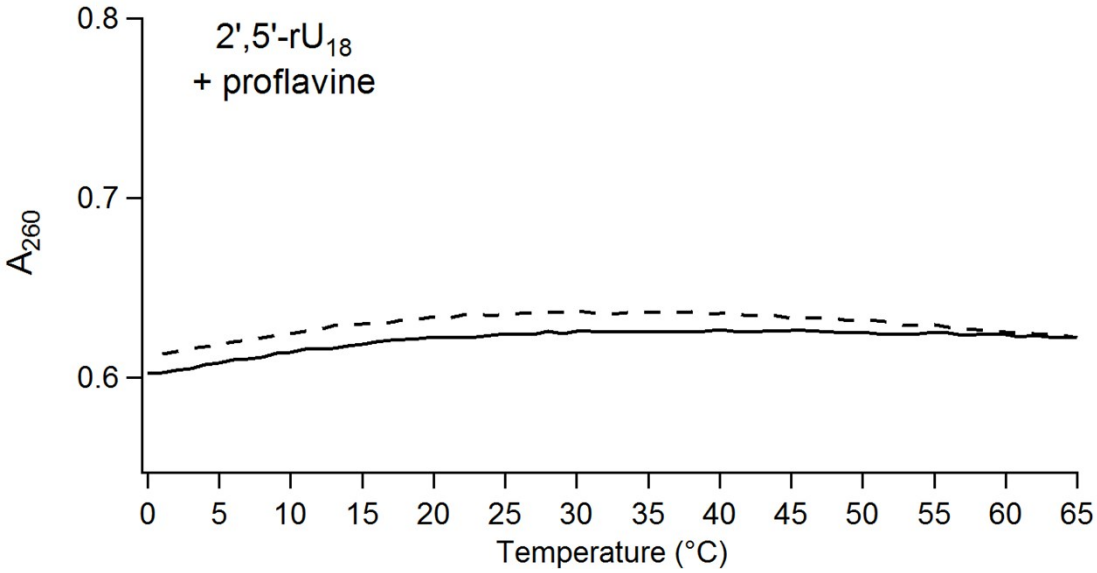

Fig. S47 UV-monitored thermal profiles of a solution containing 2',5'-rU_{18} (5 μM in oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

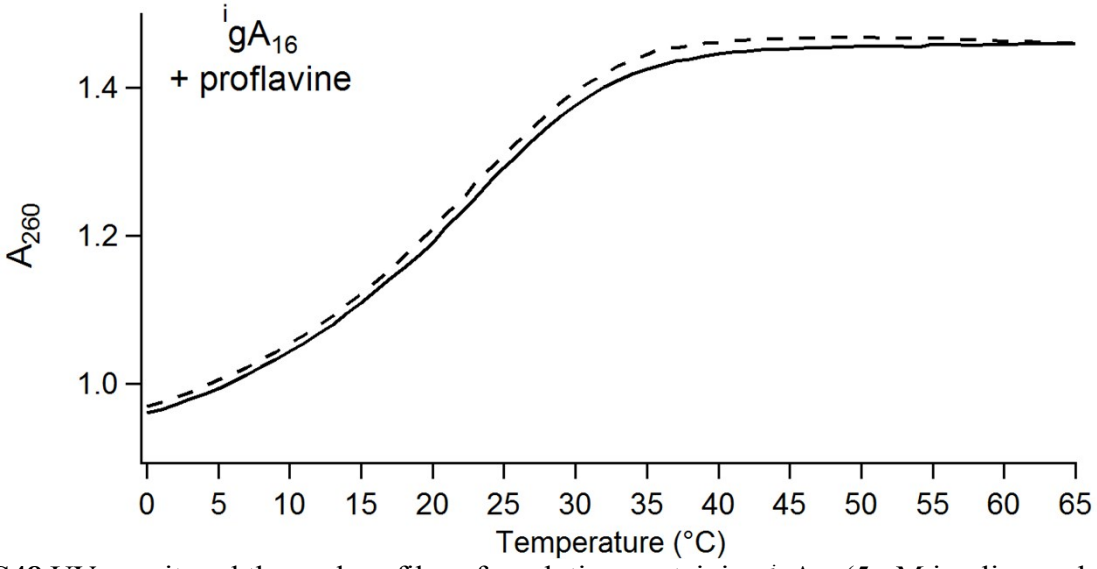

Fig. S48 UV-monitored thermal profiles of a solution containing $^{i}gA_{16}$ (5 μM in oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Note that $^{i}gA_{16}$ forms a self-structure with proflavine with a T_m of 20 °C, however, as indicated by the data in Figure S29, the duplex formed by $^{i}gA_{16}$ and $^{i}gT_{16}$ with proflavine is more stable (> 25 °C) than the $^{i}gA_{16}$ self-structure. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.

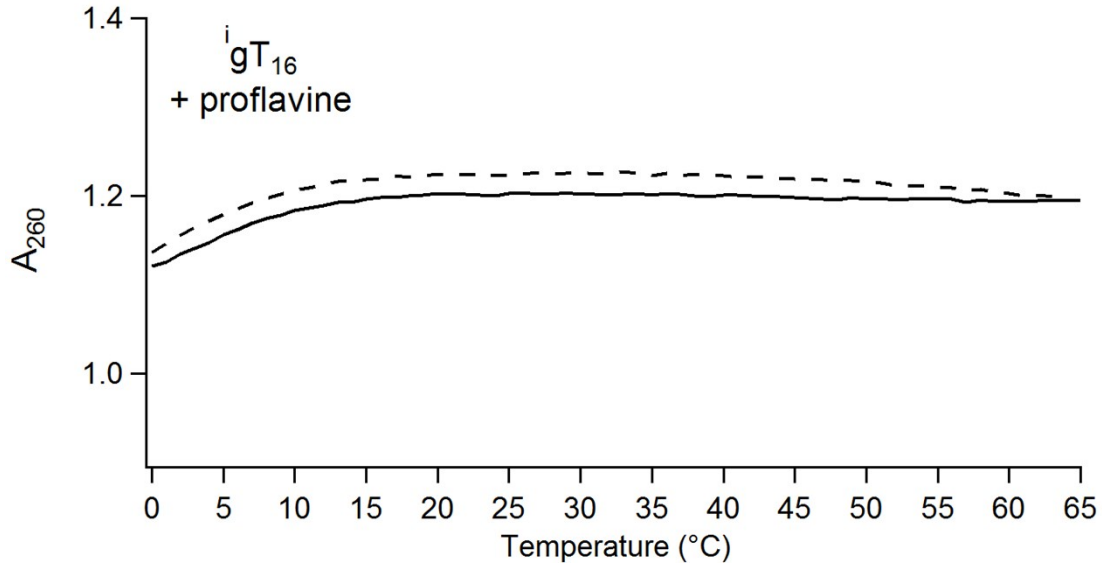

Fig. S49 UV-monitored thermal profiles of a solution containing , $^{i}gT_{16}$ (5 μM in oligonucleotide) and proflavine (45 μM). A representative heating trace is shown as a solid line and a representative cooling trace is shown as a dashed line. Solution contained 1 M NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0.