

## **Supporting Information**

### **Triple helix conformation-specific blinking of Cy3 in DNA**

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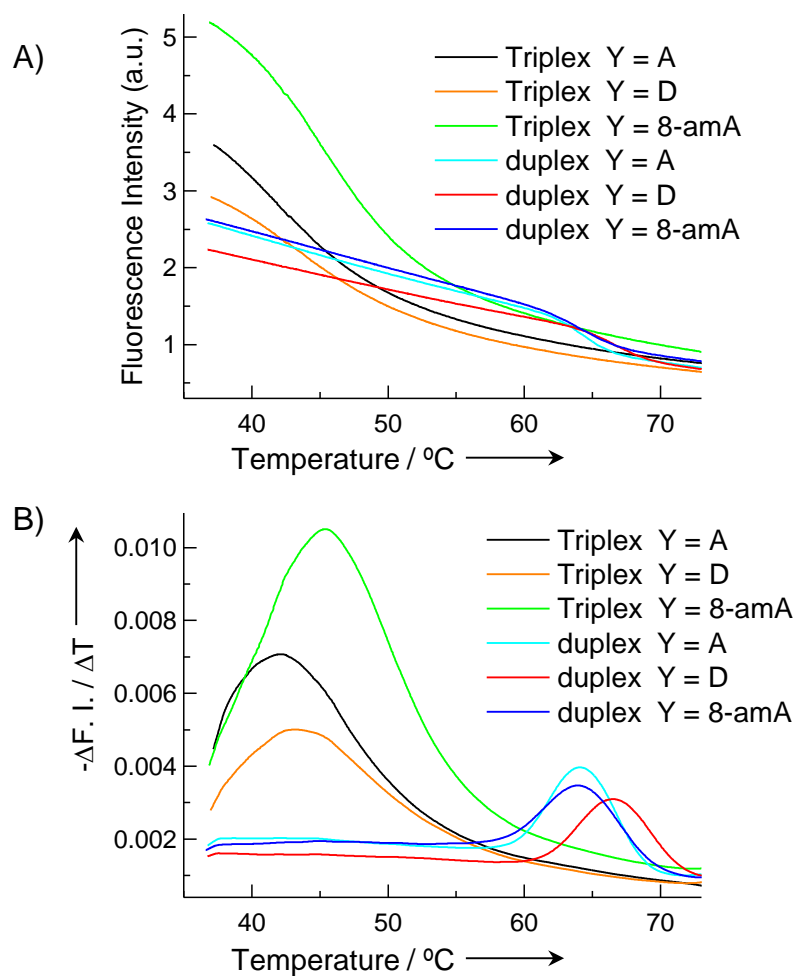
## Experimental procedures

**DNA Synthesis.** Cy3, diaminopurine, and 8-aminoadenine modified DNA were purchased from Gene Design Inc., which were synthesized according to the procedures established by our group.<sup>1,2</sup>

**Fluorescence Correlation Spectroscopy (FCS).** The FCS measurements were carried out using the MF20 (Olympus)<sup>1,3</sup> in an aqueous solution contained 4 nM Cy3 modified-DNA, 100 mM NaCl, 10 mM MgCl<sub>2</sub>, 7.5% or 15% PEG-20,000 in 10 mM Na phosphate buffer (pH 7.0). 8 nM of complementary strand was added in the case of single strand, and double helix. He-Ne laser (543 nm, 100 μW) was used as the excitation source. All experiments were performed with 10 s of data acquisition time per measurement, and repeated 4-8 times per sample.

**Melting temperature measurements.** The thermal denaturation profile was recorded on a Roche real-time PCR (LightCycler® 96). The fluorescence of the DNA sample (at a strand concentration of 4 nM in 100 mM NaCl, 10 mM MgCl<sub>2</sub>, 7.5% or 15% PEG-20,000, 10 mM sodium phosphate, (pH 7.0), with 8 nM of complementary strand in the case of double helix (same conditions as used in FCS) was monitored at 572 nm (excitation at 533 nm) from 37 to 87 °C with a heating rate of 1 °C/min. The  $T_m$  value was determined as the maximum in a plot of  $\Delta I_{572}/\Delta T$  versus temperature, and repeated 3 times per sample.

## Melting temperature ( $T_m$ ) measurement



**Figure S1.** The fluorescence melting-temperature ( $T_m$ ) was measured at 573 nm (excitation at 533 nm) corresponding to the fluorescence of Cy3.

## References

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(b) K. Kawai, T. Majima and A. Maruyama, *ChemBioChem*, 2013, **14**, 1430;  
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- 2 K. Kawai, I. Saito and H. Sugiyama, *Tetrahedron Lett.*, 1998, **39**, 5221.
- 3 S. W. Choi, A. Kano and A. Maruyama, *Nucleic Acids Res.*, 2008, **36**, 342.