Supporting information for:

A pH-driven DNA switch based on the A+•G mispair

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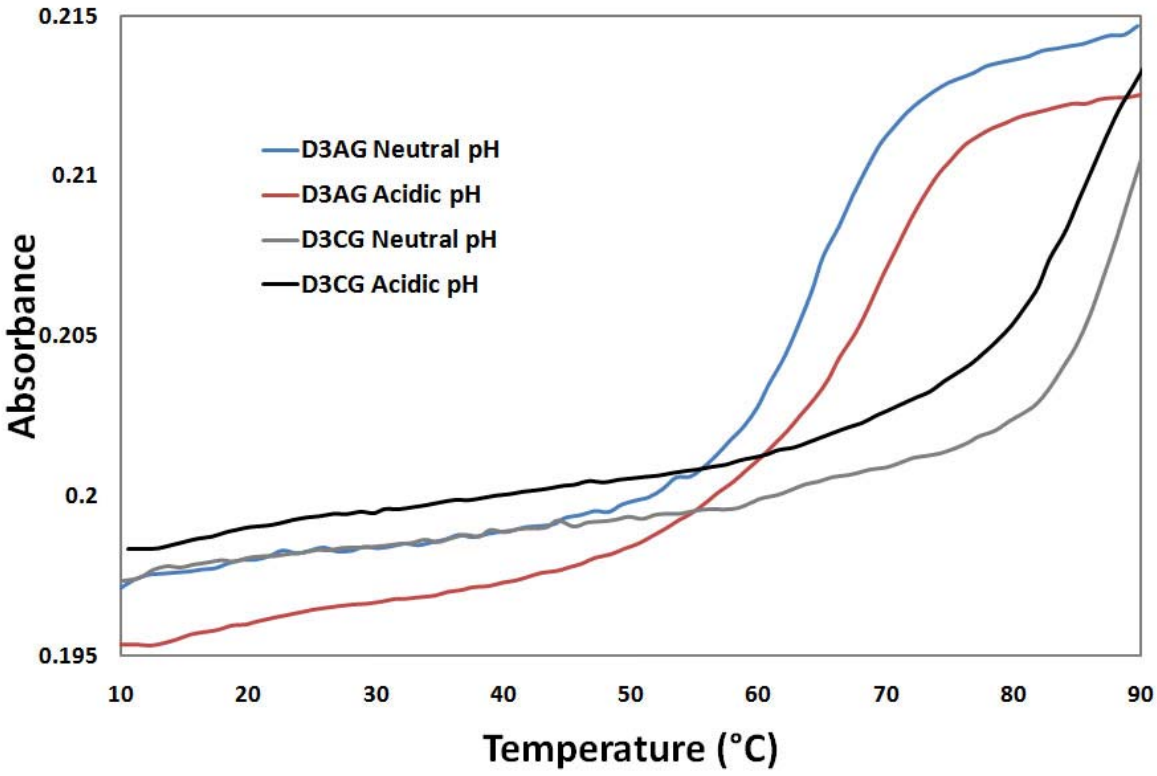

Figure S1: Melting temperature experiments of D3AG and D3CG under neutral and acidic conditions. Although data from D3CG could not be fit using the Origin software, it can be seen from the absorbance data that it has a higher melting temperature under neutral conditions, as was found with the other control sequences.

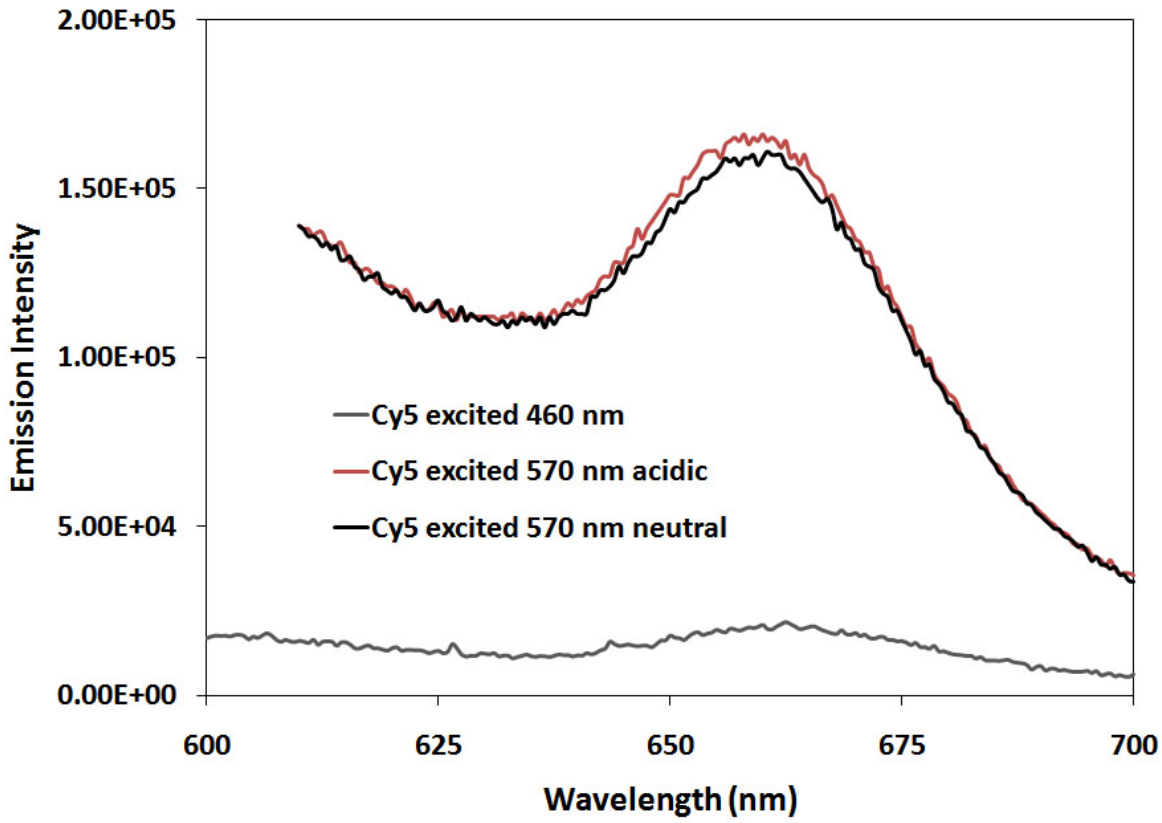

Figure S2: Controls with D3AG labeled with Cy5 only (5'-end). Grey line: Emission spectrum after excitation at 460 nm, the excitation wavelength used in pH cycling experiments. Minimal emission is detected at 662 nm. Black line: Emission spectrum after excitation at 570 nm (neutral pH). Red line: Emission spectrum after excitation at 570 nm (acidic pH). The intensity of the Cy5 emission changes very little as a function of pH. Spectra taken at 60°C.

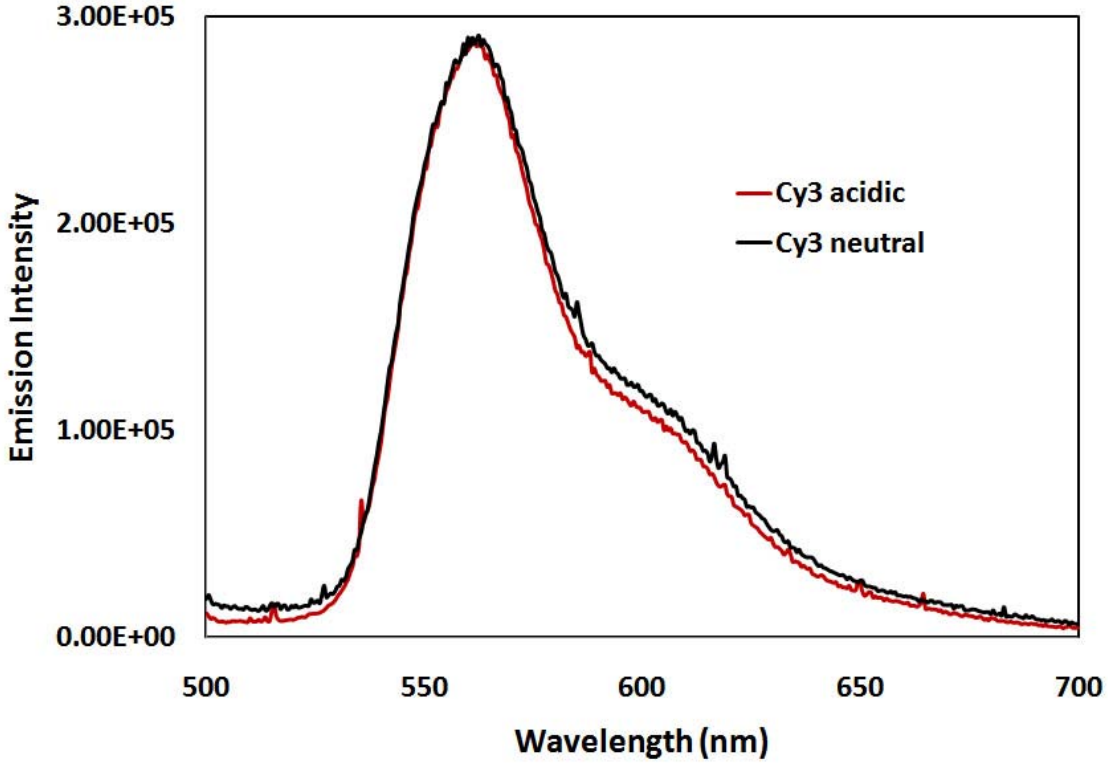

Figure S3: Controls with D3AG labeled with Cy3 only (3'-end). Black line: Emission spectrum at neutral pH (excitation 460 nm). Red line: Emission spectrum at acidic pH (excitation at 460 nm). The intensity of the Cy3 emission changes very little as a function of pH. Spectra taken at 60°C.

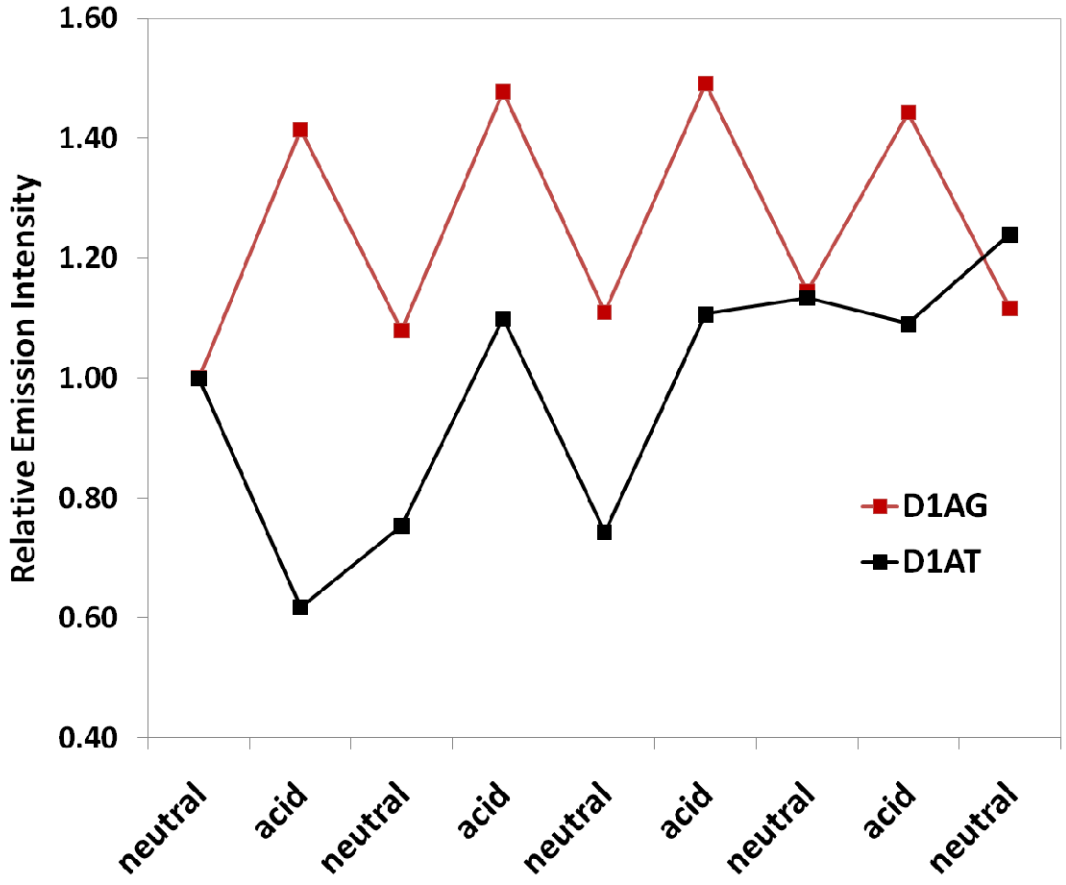

Figure S4: Comparison of pH switching experiments with D1AG and D1AT, both doubly labeled (Cy3 – 3', Cy5 – 5'). Experiments were run at 37°C. D1AT shows no trend in emission intensity as it is cycled from neutral to acidic pH.

Materials and Methods:

Unmodified DNA sequences were prepared on the 1 µmol scale with a Bioautomation MerMade DNA synthesizer using standard phosphoramidite chemistry. DNA sequences were purified on an Agilent 1200 HPLC with a Zorbax 300 Ext – C18 reverse phase column. Cy3 and Cy5 singly and doubly labeled oligonucleotides (Cy3 labeling on the 3'-end, Cy5 labeling on the 5' end) were purchased HPLC purified from Alpha DNA.

Melting temperature studies were done on a Cary 300 Bio UV-Visible spectrophotometer with a Varian temperature controller. Oligonucleotide sequences (concentration of 0.1-0.3 AO) in both acetate buffer (0.1 M, pH 5) and phosphate buffer (0.1 M, 50 mM NaCl, pH 7) were heated to 90ºC and allowed to cool to room temperature. Absorbance at 260nm was recorded at 1ºC intervals from 15-90°C and the data was fit to a Boltzmann sigmoidal curve using OriginPro8.

pH cycling experiments were done on a Horiba Jobin Yvon Fluorolog fluorometer equipped with a Wavelength Electronics temperature controller. Labelled DNA sequences were dissolved in 3 mL of 1 mM phosphate buffer (pH 7, 0.2 µM DNA, 50 mM NaCl). Small aliquots of 1.2M HCl (2.1 µL) or 1.25M NaOH (2.25 µL) were added during pH cycling experiments to avoid diluting the sample or dramatically changing the ionic strength. After stirring for 2 minutes, the pH was measured inside the fluorescence cell using a Lazar micro pH electrode and the emission spectra from 500-700 nm were recorded (excitation 460 nm).