

## Supporting Information for

# Red-White-Blue Emission Switching Molecular Beacons: Ratiometric Multicolour DNA Hybridization Probes

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## 1. Experimental Section

### General

Chemicals and dry solvents were purchased from commercial suppliers and were used without further purification unless otherwise mentioned. TLC was performed on Fluka silica gel 60 F254 coated aluminium foil. Flash chromatography was carried out with Silica Gel 60 from Aldrich (60-43  $\mu\text{m}$ ). Spectroscopic measurements were recorded in Na-Pi buffer solution (10 mM) using quartz glass cuvettes ( $l = 10$  mm). ESI mass spectra were measured in the central analytical facility of the institute on a ThermoQuest Finnigan TSQ 7000 in negative ionisation mode. NMR spectra were recorded on a Bruker Avance 300 spectrometer in deuterated solvents ( $^1\text{H}$  at 300 MHz,  $^{13}\text{C}$  at 75 MHz and  $^{31}\text{P}$  at 121.5 MHz). Absorption spectra and the melting temperatures (1  $\mu\text{M}$  DNA, 10-80  $^{\circ}\text{C}$ , 0.7  $^{\circ}\text{C}/\text{min}$ , step width 0.5  $^{\circ}\text{C}$ ) were recorded on a Varian Cary 100 spectrometer equipped with a 6 $\times$ 6 cell changer unit. Fluorescence was measured on a Jobin-Yvon Fluoromax 3 fluorimeter with a step width of 1 nm and an integration time of 0.2 s. All spectra were recorded with an excitation and emission bandpass of 5 nm and are corrected for Raman emission from the buffer solution. Phosphoramidite of ethynyl pyrene was commercially available (Glen research) and the corresponding phosphoramidite of Nile red was synthesized according to our recent publication.<sup>1</sup>

## Synthesis of Molecular Beacons

The oligonucleotides were prepared on an Expedite 8909 DNA synthesizer (Applied Biosystems) via standard phosphoramidite protocols using CPGs (1  $\mu\text{mol}$ ) with a longer coupling time of 15 minutes and a higher concentration of the phosphoramidite (0.1 M). The chemicals for the DNA synthesis were purchased from ABI and Glen Research. Unmodified oligonucleotides were purchased from Metabion. After preparation, the trityl-off oligonucleotide was cleaved off the resin and was deprotected by treatment with conc.  $\text{NH}_4\text{OH}$  at RT for 15 h. The oligonucleotides were dried and purified by reverse phase HPLC using the following conditions: A =  $\text{NH}_4\text{OAc}$  buffer (50 mM), pH = 6.5; B = MeCN; gradient = 0-20% B over 50 min for **DNA1-3** and 0-30% B over 50 min for **DNA4**. The oligonucleotides were lyophilized and quantified by their absorbance at 260 nm on a Varian Cary Bio 100 spectrometer. Self-hybridized duplexes (hairpins) were prepared by heating the corresponding DNAs to 90 °C (hold for 10 min.) followed by immediate cooling using an ice bath or liquid nitrogen. Duplexes with the target of interest were prepared by simply mixing ca. 1 equivalence of the target DNA with hairpin MB at room temperature.

**Table S1.** m/z Values of ss-**DNA1-4** determined by ESI-MS.

DNA	Calculated mass (m/z)	Observed mass (m/z)
<b>DNA1</b>	11289.8	11289.0
<b>DNA2</b>	11280.8	11281.2
<b>DNA3</b>	11271.8	11272.2
<b>DNA4</b>	8522.3	8521.0

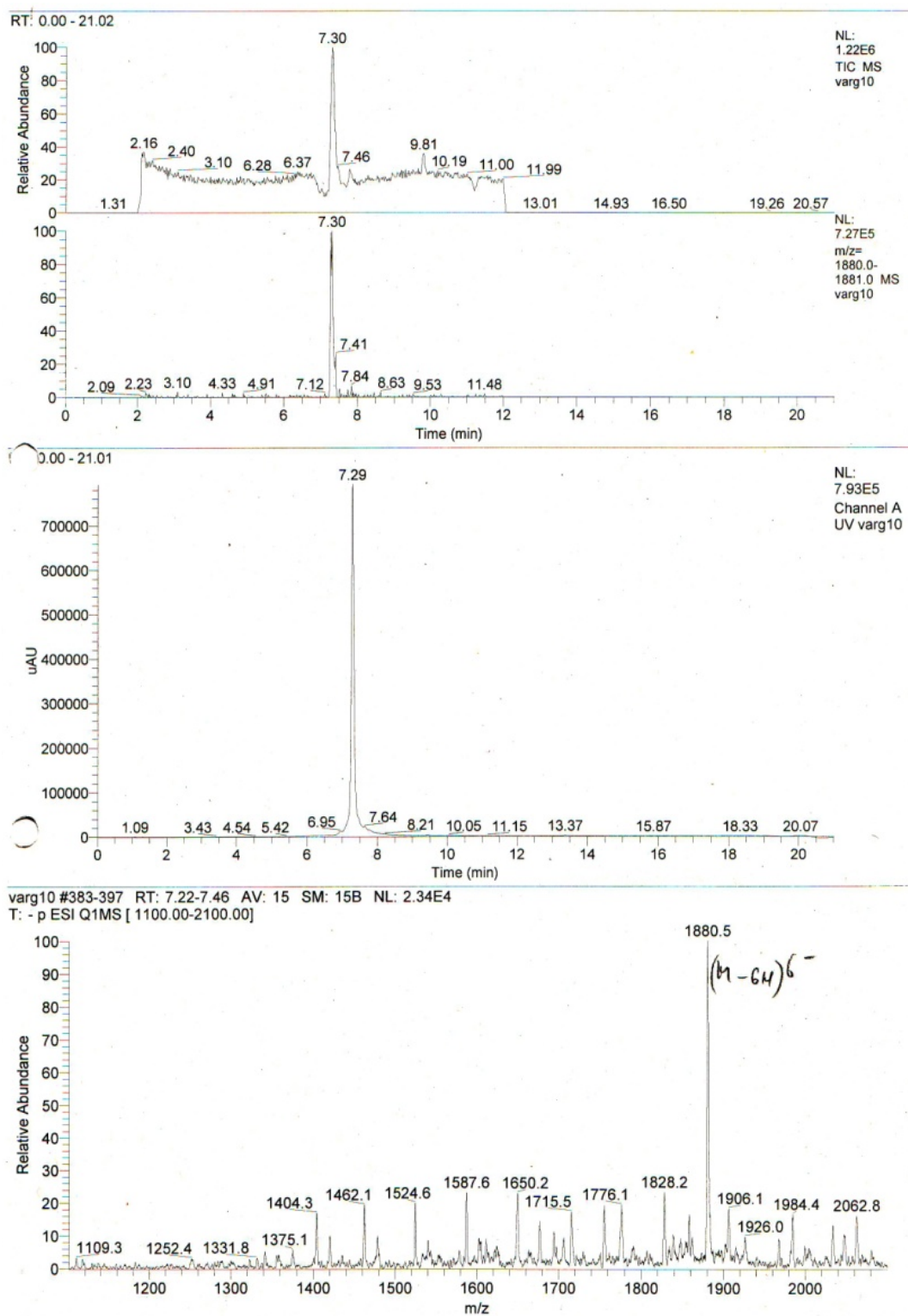


Figure S1. LC-MS of DNA1.

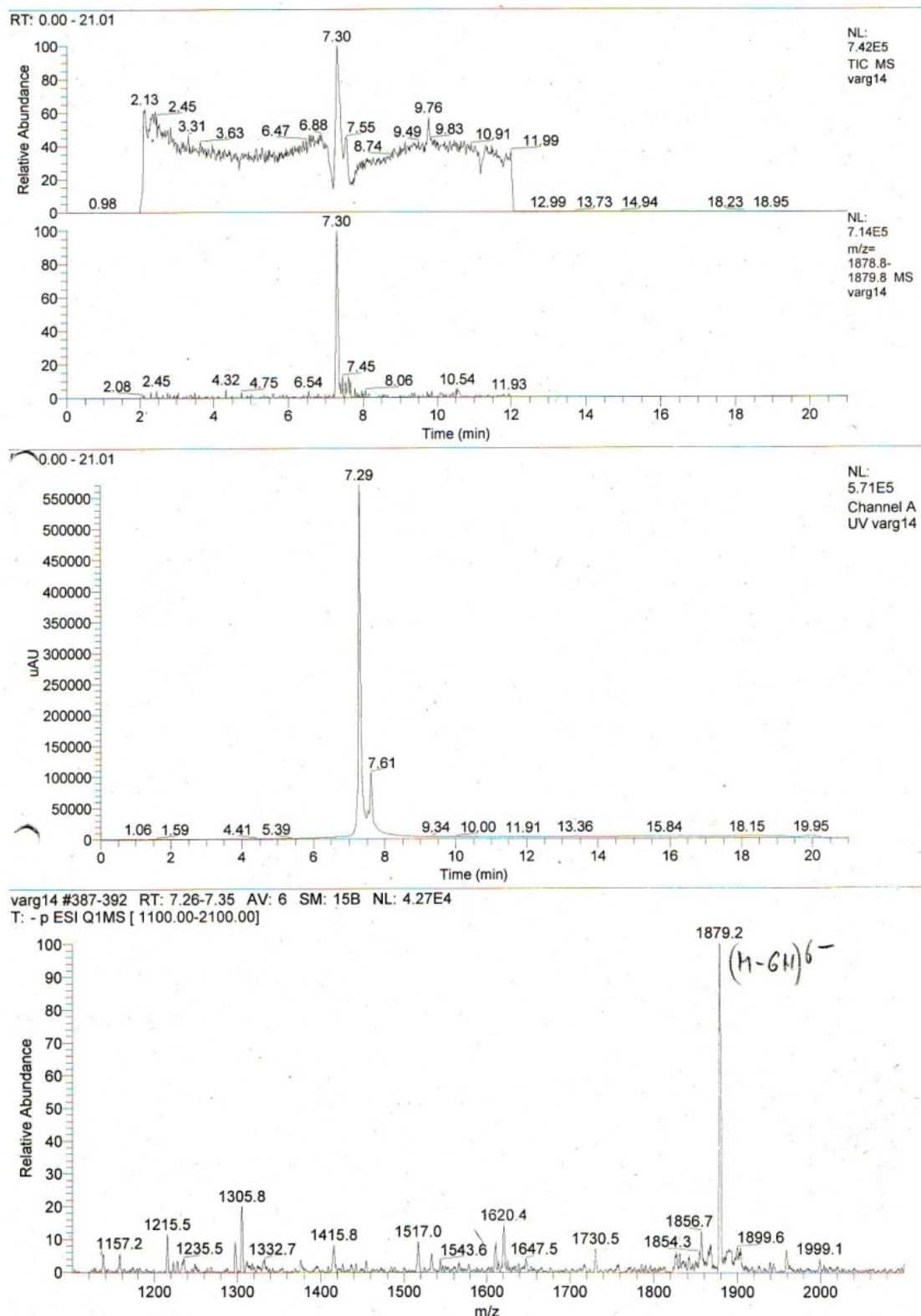


Figure S2. LC-MS of DNA2.

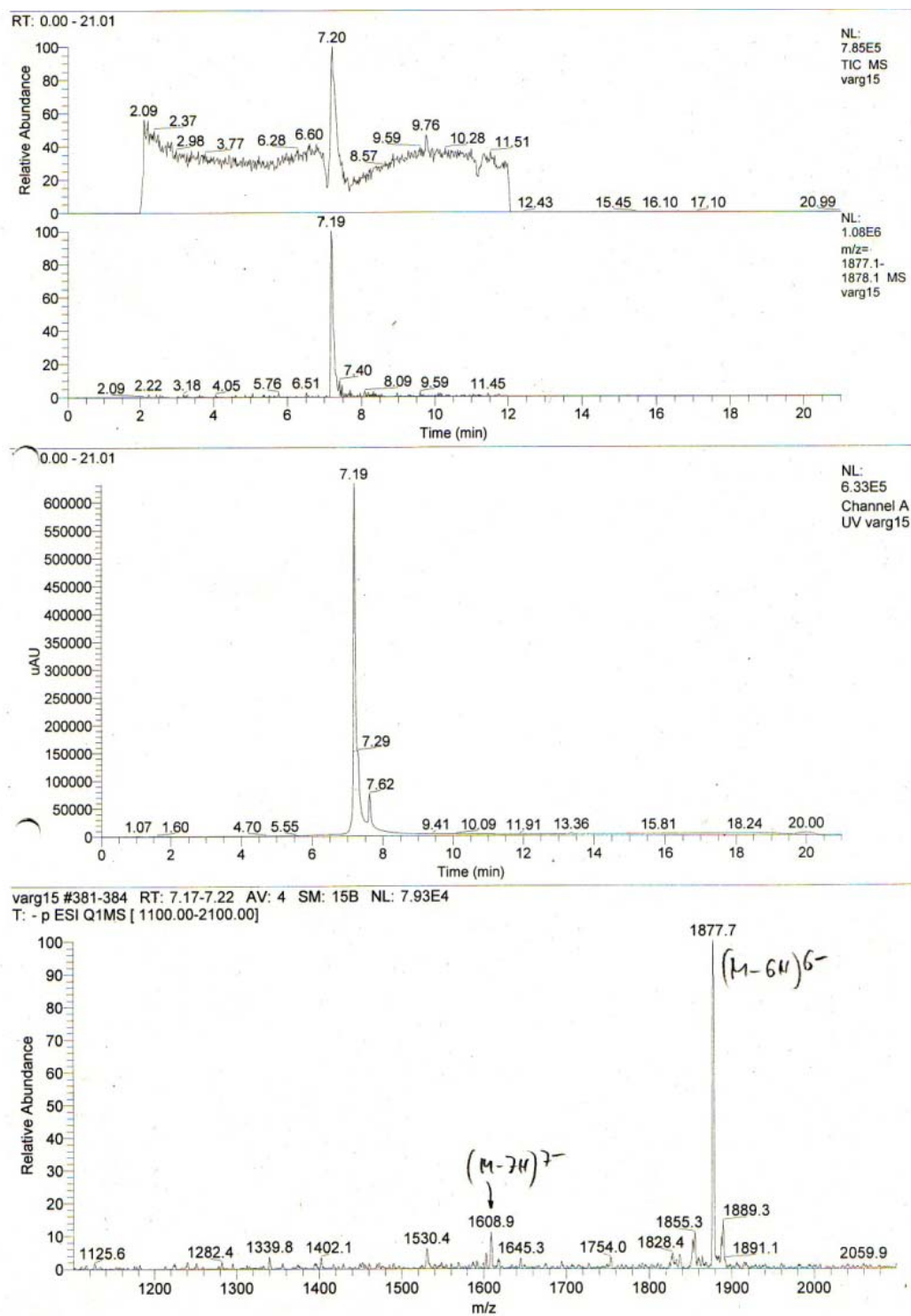
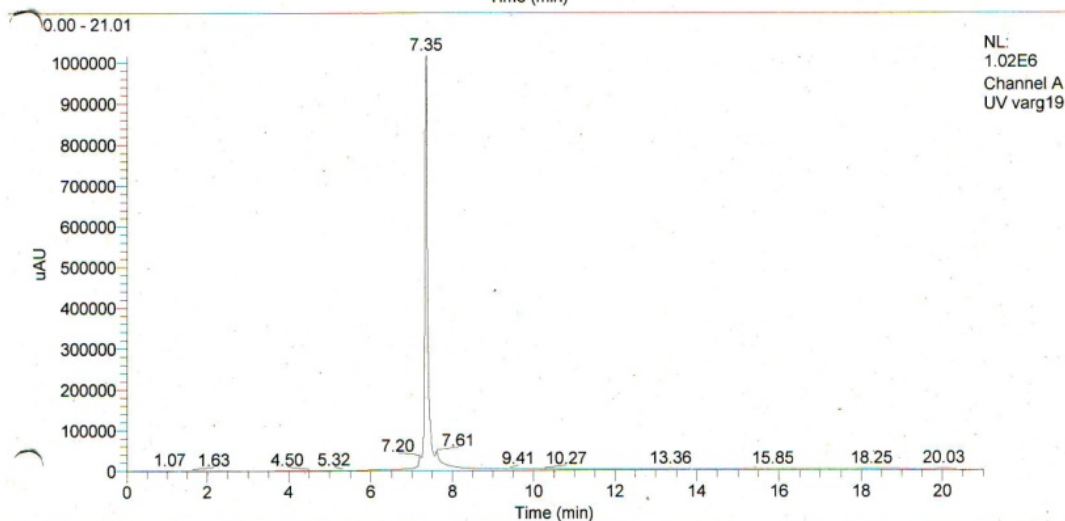
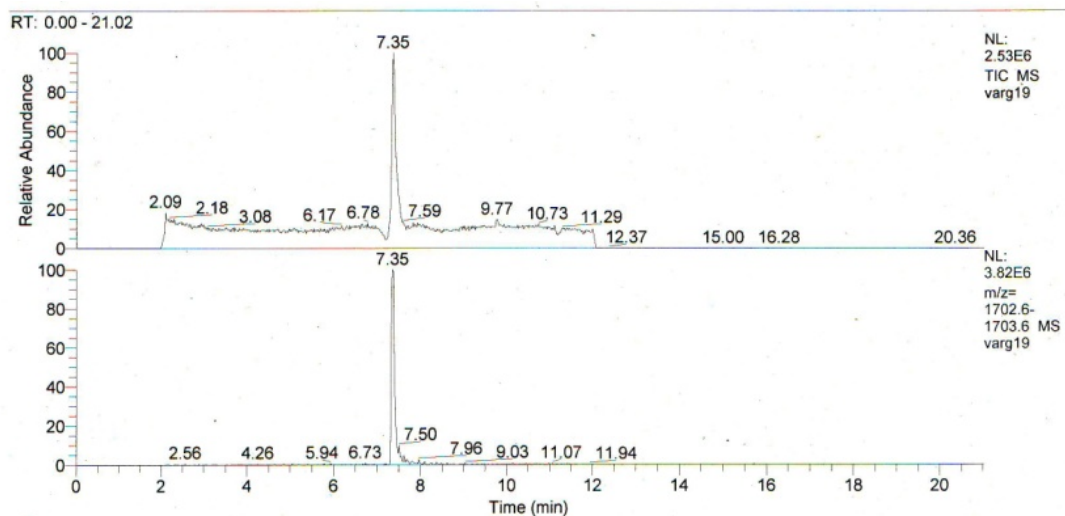
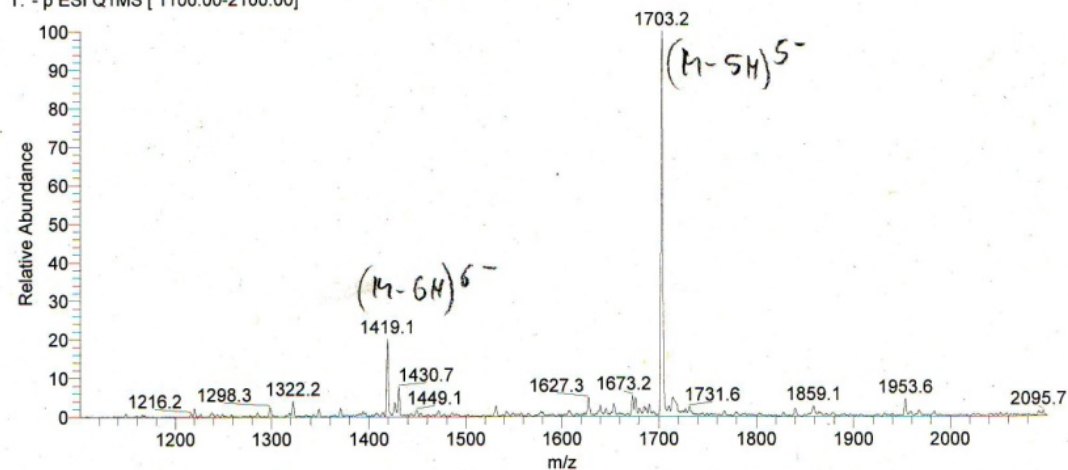


Figure S3. LC-MS of DNA3.

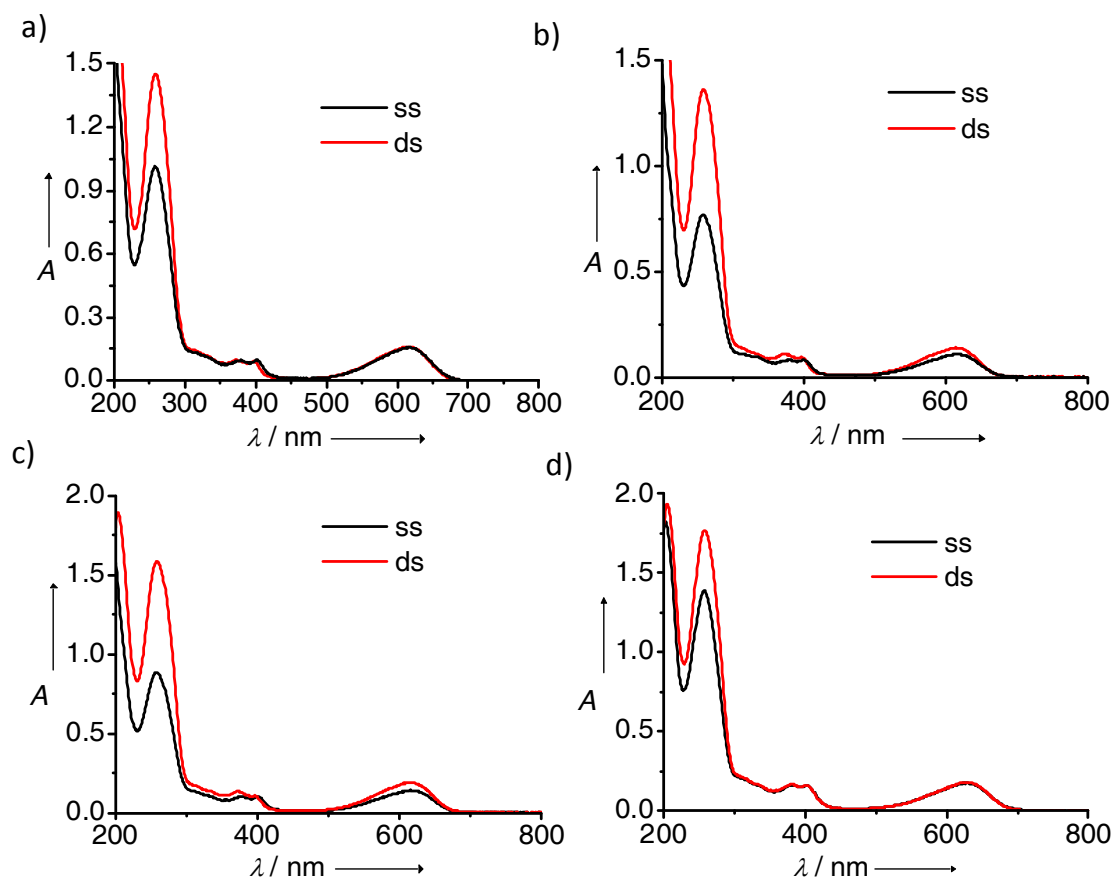


varg19 #389-399 RT: 7.29-7.45 AV: 11 SM: 15B NL: 1.68E5  
T: -p ESI Q1MS [1100.00-2100.00]

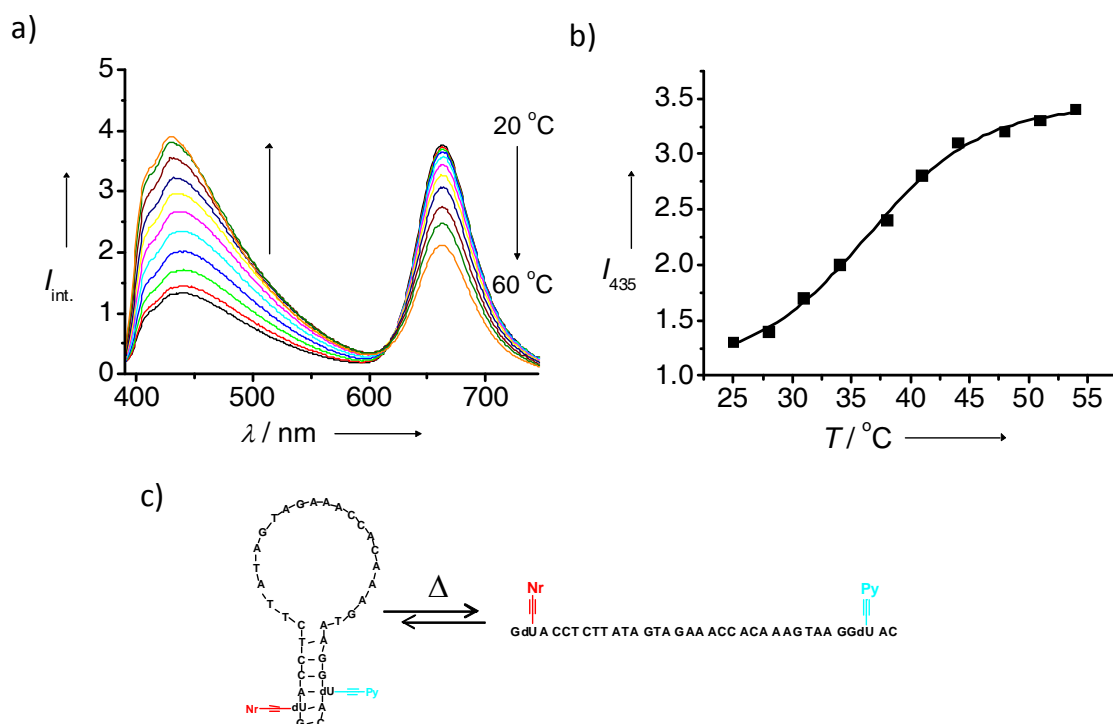




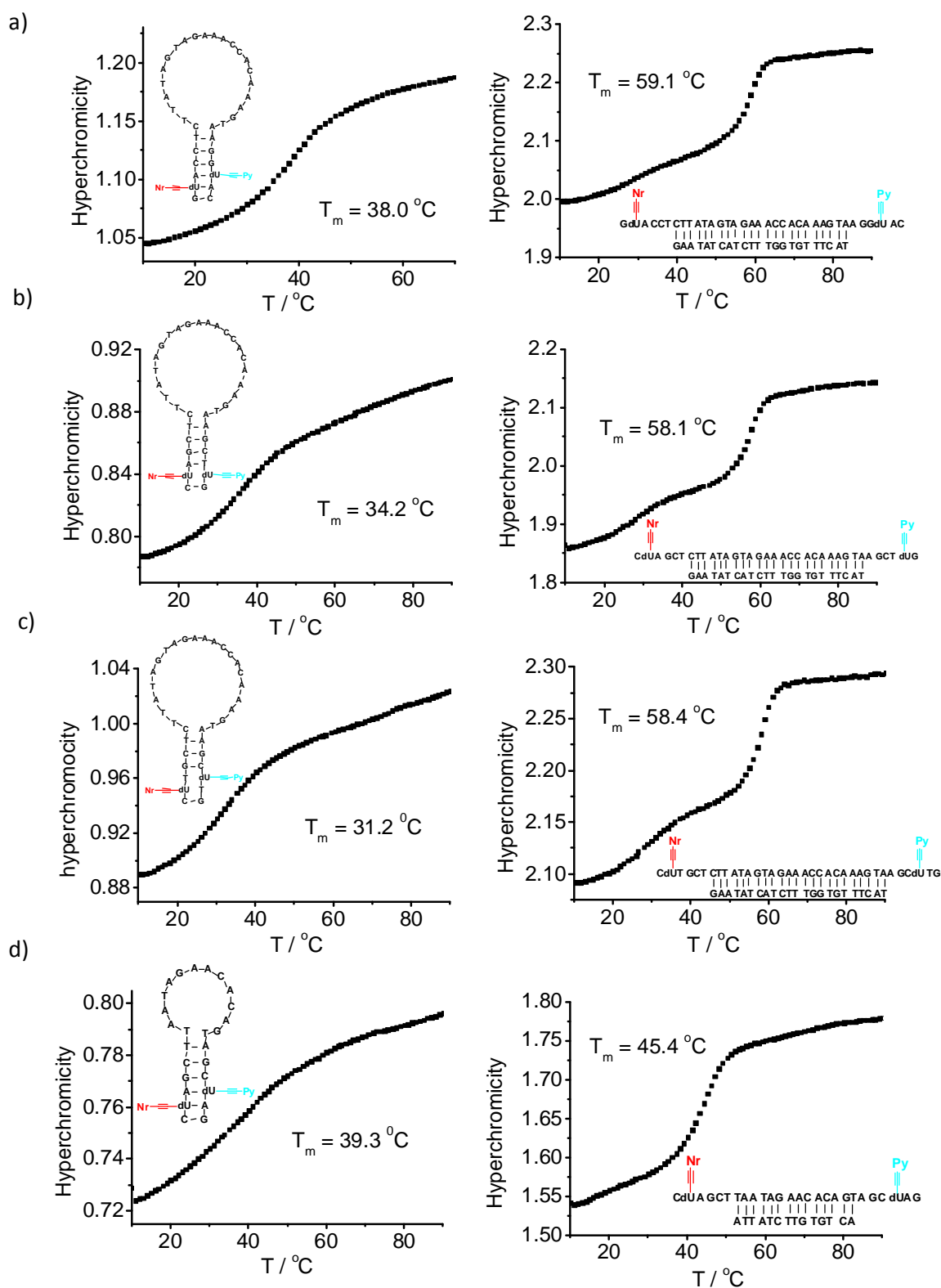
**Figure S4. LC-MS of DNA4.**



**Figure S5:** Absorption spectra of ss- and ds-DNAs, a) **DNA1**, b) **DNA2**, c) **DNA3** and d) **DNA4** ( $c = 1 \mu\text{M}$  in Na-*Pi* buffer, 250 mM NaCl,  $\text{pH} = 7$ ,  $T = 25 \text{ }^\circ\text{C}$ ).

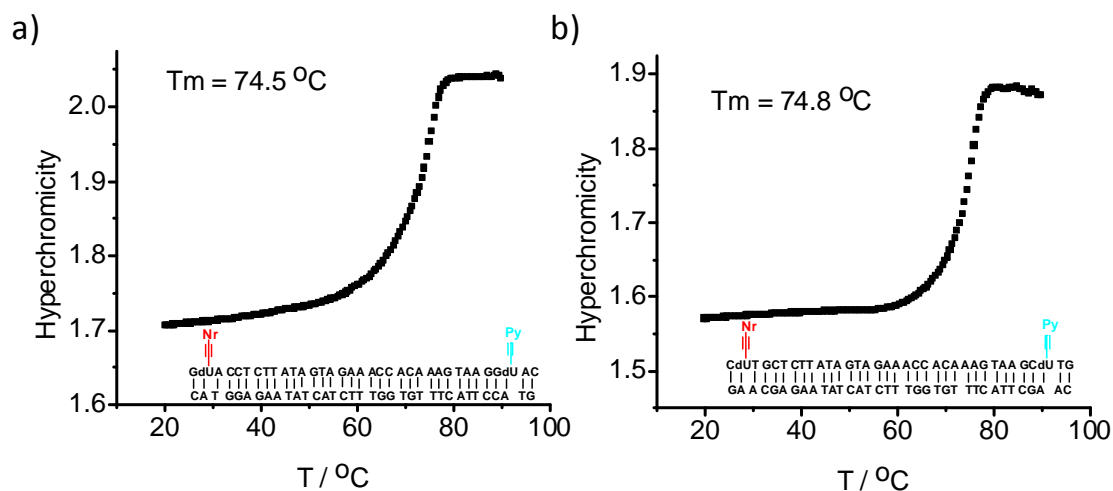


**Figure S6:** a) Temperature dependent fluorescence spectra of **DNA1** and b) plot of  $I_{435}$  of **DNA1** against temperature. ( $c = 1 \mu\text{M}$  in Na-Pi buffer, 250 mM NaCl, pH = 7,  $\lambda_{exc} = 380$  nm,  $T = 25 \text{ }^\circ\text{C}$ ). A melting temperature of  $\approx 40 \text{ }^\circ\text{C}$  is observed for the melting of the hairpins of **DNA1**. c) A pictorial representation of the temperature dependent association and dissociation of the hairpin.

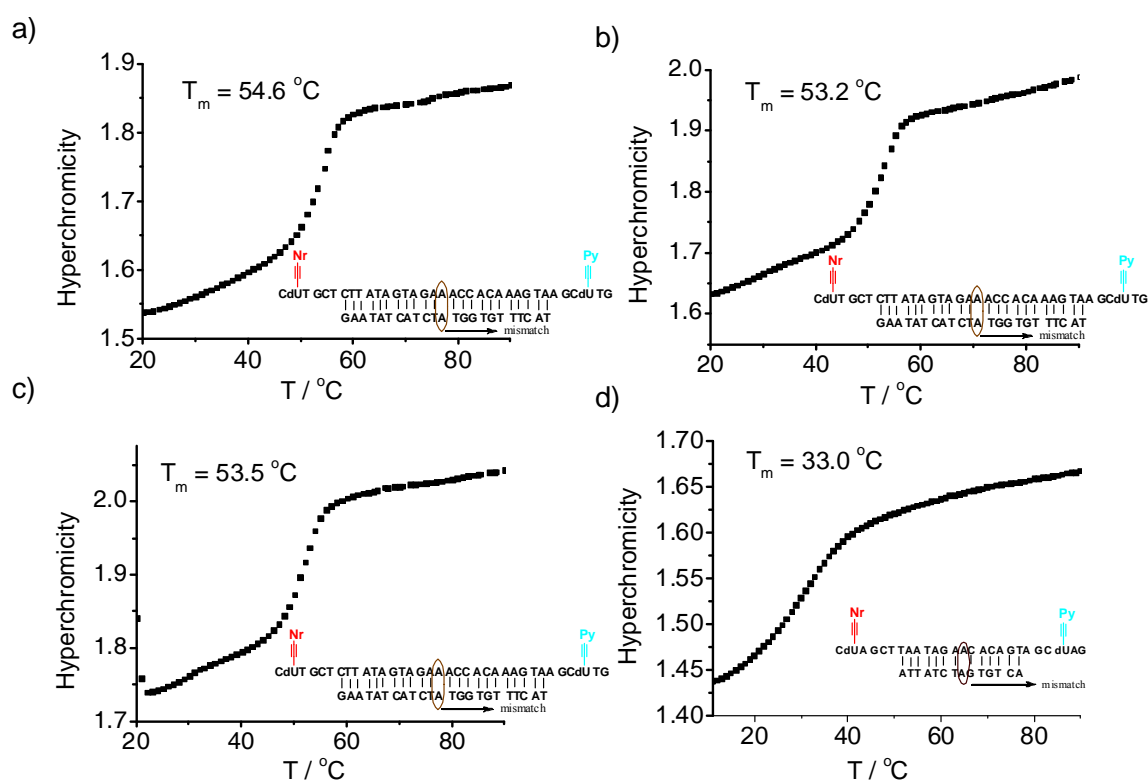


**Figure S7.** Melting curves for hairpins (left) and duplexes (right) monitored at 260 nm (heating cycle); a) DNA1, b) DNA2, c) DNA3, d) DNA4 ( $c = 1 \mu\text{M}$  in Na-Pi buffer, 250

mM NaCl, pH= 7). The weak transition observed in the temperature range of 20-40 °C in the duplexes of **DNA1-4** may be attributed to the melting of the duplex formed through the “sticky end” association<sup>2</sup> ( $c = 1 \mu\text{M}$  in Na-Pi buffer, 250 mM NaCl, pH = 7).



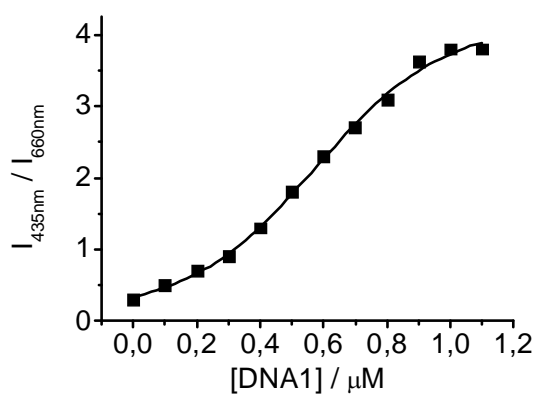
**Figure S8.** Melting curves for the duplexes of a) **DNA1** and b) **DNA3** with full length complementary strands (5'-CATGGAGAATATCATCTTTGGTGTTTCATTCCATG-3' and 5'-GAACGAGAATATCATCTTTGGTGTTTCATTCGAAC-3' for **DNA1** and **DNA3**, respectively). Absorbance changes are monitored at 260 nm ( $c = 1 \mu\text{M}$  in Na-Pi buffer, 250 mM NaCl, pH= 7). This experiment is suggested by one of the reviewer during the revision of the paper in order to prove the weak transition appeared in the melting of the duplex in Figure S7 corresponds to the melting of the duplex formed through the “stick end” association. The disappearance of the weak transition in this case clearly supports the proposed hypothesis.



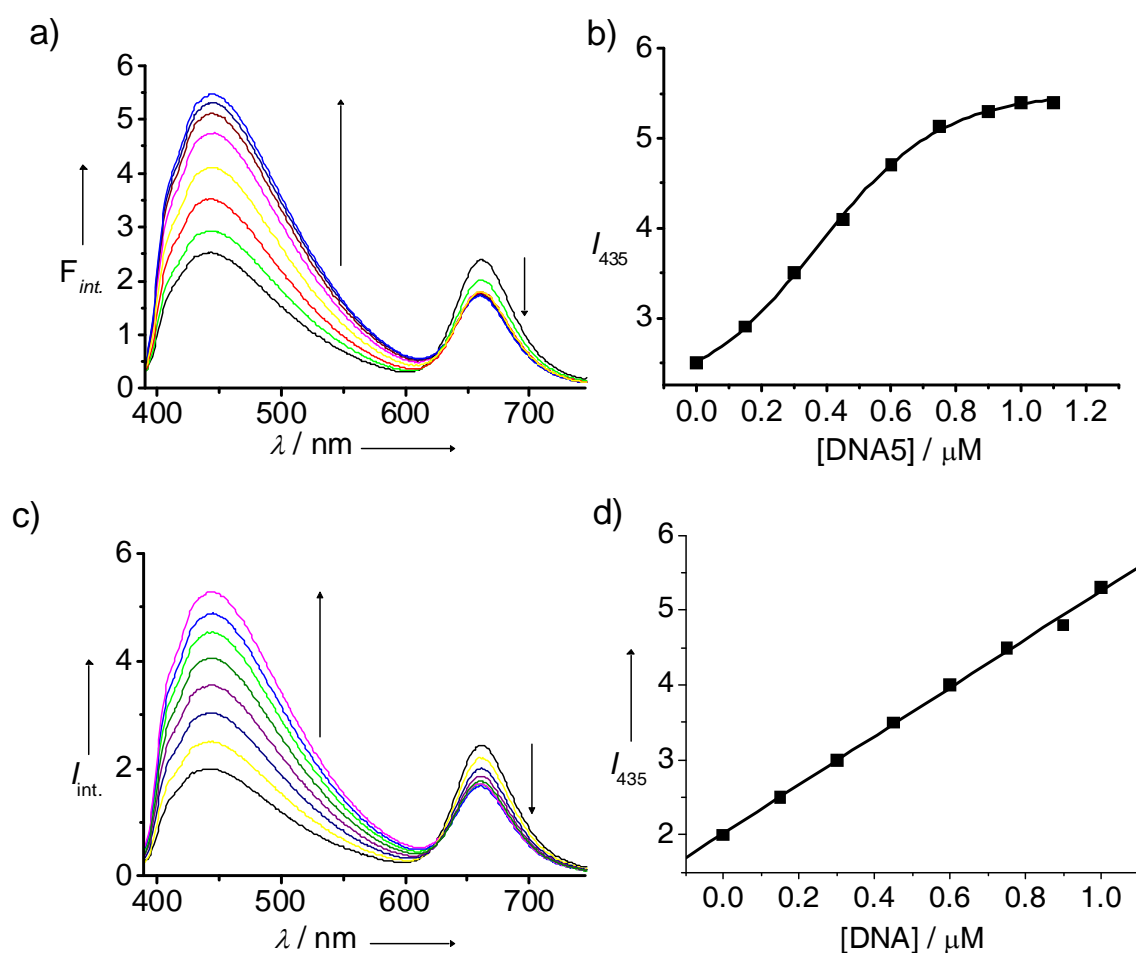
**Figure S9.** Melting curves of the duplex of **DNA1-4** with target DNAs contains SNP monitored at 260 nm (heating cycle); a) **DNA1**, b) **DNA2**, c) **DNA3**, d) **DNA4**.

**Table S2.** Melting temperatures of duplex **DNA1-4** with matched targets and targets with SNP.  $\Delta T$  represents the difference in melting temperatures between duplex and hairpin.

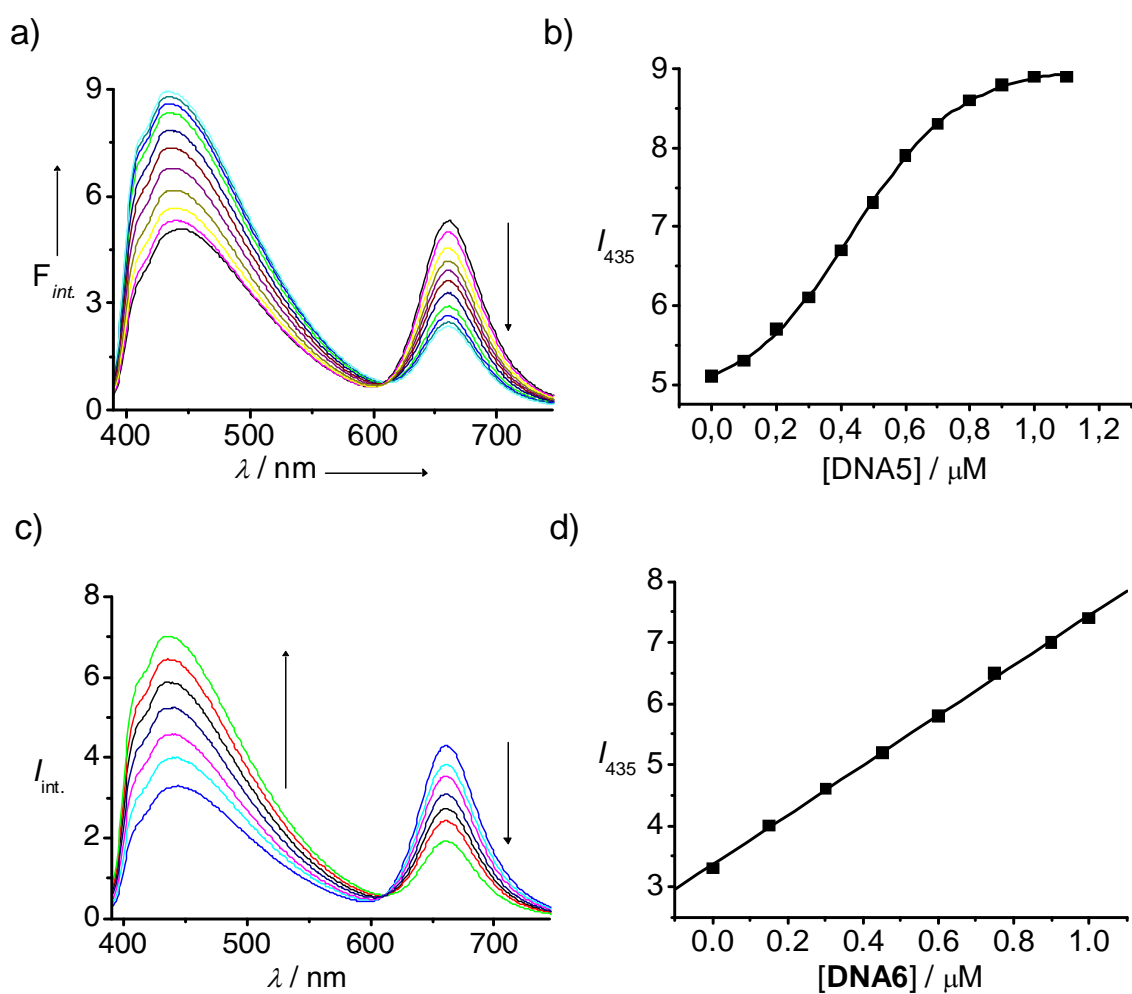
DNA	Matched target			Mismatch target	
	T <sub>m</sub> (hairpin) (°C)	T <sub>m</sub> (duplex) (°C)	$\Delta T$ (°C)	T <sub>m</sub> (hairpin) (°C)	$\Delta T$ (°C)
<b>DNA1</b>	38.0	59.1	21.1	54.6	16.6
<b>DNA2</b>	34.2	58.1	23.9	53.2	19.0
<b>DNA3</b>	31.2	58.4	27.2	53.5	22.3
<b>DNA4</b>	39.3	45.4	6.1	33.0	-6.3



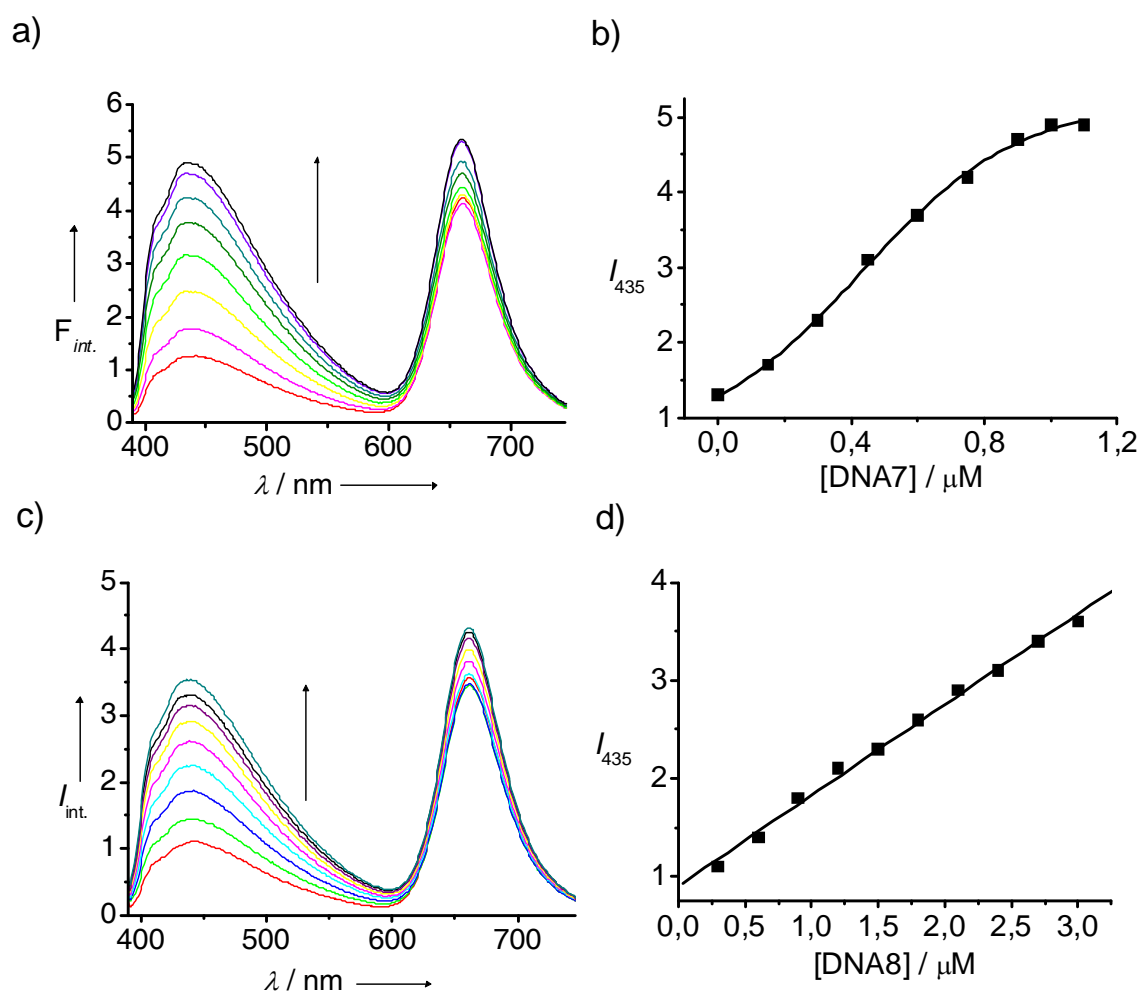
**Figure S10.** Plot of  $I_{435}/I_{660}$  of **DNA1** against the concentration of **DNA5** ( $c = 1 \mu\text{M}$  in Na-Pi buffer, 250 mM NaCl, pH = 7).



**Figure S11.** Fluorescence titration spectra of **DNA2** with a) **DNA5** and c) **DNA6**. Plot of  $I_{435}$  against the concentration of b) **DNA5** and d) **DNA6** ( $c = 1 \mu\text{M}$  in Na-Pi buffer, 250 mM NaCl, pH = 7).



**Figure S12.** Fluorescence titration spectra of **DNA3** with a) **DNA5** and c) **DNA6**. Plot of  $I_{435}$  against the concentration of b) **DNA5** and d) **DNA6** ( $c = 1 \mu\text{M}$  in Na-Pi buffer, 250 mM NaCl, pH = 7).



**Figure S13.** Fluorescence titration spectra of **DNA4** with a) **DNA7** and c) **DNA8**. Plot of  $I_{435}$  against the concentration of b) **DNA7** and d) **DNA8** ( $c = 1 \mu\text{M}$  in Na-Pi buffer, 250 mM NaCl, pH = 7). **DNA8** = ACTGTGATCTATTA (5'→3') (underline indicates a mismatch).

### References:

1. R. Varghese and H.-A. Wagenknecht, *Chem. Eur. J.*, **2009**, *15*, 9307.
2. I. V. Nesterova, S. S. Erdem, S. Pakhomov, R. P. Hammer and S. A. Soper, *J. Am. Chem. Soc.*, **2009**, *131*, 2432.