

Detection of a single DNA base-pair mismatch using an anthracene-tagged fluorescent probe†

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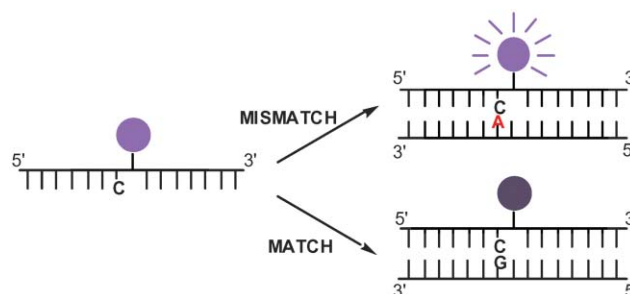
A novel anthracene-tagged oligonucleotide can discriminate between a fully-matched DNA target sequence and one with a single mismatching base-pair through a remarkable difference in fluorescence emission intensity upon duplex formation.

The accurate detection of single-nucleotide polymorphisms (SNPs) in DNA is the subject of intense research. SNPs are thought to be the cause of genetic disorders (most human genetic variations result from SNPs) and thus a fast, reliable and inexpensive sensor is needed for use in DNA diagnostics.¹ Of the many luminescence-based methods used for the detection of mismatches in DNA,² one of the simplest is to tag a single fluorophore to an oligonucleotide probe and monitor changes in its emission intensity upon duplex formation. By tagging pyrene to DNA in this manner, particularly promising SNP sensors have been developed.³

In contrast to pyrene, only a few examples of anthracene-tagged oligonucleotides have been reported.⁴ This is in spite of the well-characterized properties of anthracene, these being its ability to form excimers and undergo $[4\pi + 4\pi]$ photocycloaddition, making it a versatile and useful fluorophore.⁵ Here we report an anthracene-tagged SNP sensor that can discriminate between fully matched (cognate) and single base-pair mismatched target DNA sequences through quenching and enhancement of fluorescence emission respectively (Scheme 1).

Previous work on pyrene-based SNP sensors³ has involved tagging the chromophore to a nucleobase. Here a non-nucleosidic unit **5**,⁶ containing a serinol linker previously identified as an effective substitute for one nucleoside,⁷ was synthesized *via* the route shown^{5c} in Scheme 2.†

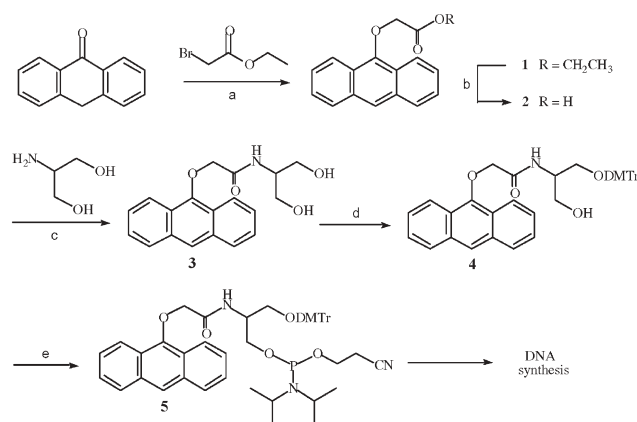
Thus ester **1** was prepared from anthrone and ethyl bromoacetate. Subsequent saponification yielded the corresponding anthracene carboxylic acid **2** following acidification.



Scheme 1 Schematic drawing of the SNP sensing mechanism; a matching CG pair causes quenching whereas a CA mismatch enhances emission.

Carbodiimide-mediated condensation of **2** with serinol afforded the corresponding amide **3** bearing a 1,3-bis alcohol. Using standard reaction protocols, these alcohol functions were sequentially tritylated to the mono-protected racemate **4** and then phosphitylated to afford the novel phosphoramidite **5** as a mixture of diastereomers. This was then inserted at the central position of 15-mer oligonucleotides *via* automated solid-phase synthesis applying extended DNA coupling conditions for the modification.

The two diastereomers (**a** and **b**) of Probe **1** (Table 1) could be separated by RP-HPLC and were thus separately isolated and characterised by MALDI-TOF mass spectrometry (see ESI). The six oligonucleotides synthesised for this study, including a fully



Scheme 2 Reagents and conditions: (a) K_2CO_3 , acetone, reflux, o/n, 35%; (b) i. 10% NaOH (aq) soln–EtOH (1 : 1), reflux, o/n; ii. HCl, 93%; (c) HOBt, DIPIC, DIEA, DMF, 40 °C, 40 h, 71%; (d) DMTrCl, DMAP, pyridine, rt, o/n, 42%; (e) (*i*-Pr₂N)PClO(CH₂)₂CN, DIEA, DCM, rt, 1.5 h, 76%.

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† Electronic supplementary information (ESI) available: Experimental procedures for the synthesis and characterisation of anthracene-tagged oligonucleotides and thermal melting curves. See DOI: 10.1039/b611650g

Table 1 Oligonucleotides synthesised in this study

Sequence (X = anthracene tagged unit)	
Probe 1	5'-TGGACTCXCTCAATG-3'
FM1 ^a	5'-CATTGAGAGAGTCCA-3'
1MM ^a	5'-CATTGAGAAAAGTCCA-3'
2MM ^a	5'-CATTGAGAATGTCCA-3'
Z ^a	5'-TTAGGGAATTAGCG-3'
FM2 ^a	5'-TGGACTCACTCAATG-3'

^a Purchased from Operon Biotechnologies.

matching sequence, **FM1** and sequences **1MM** and **2MM** with one and two mismatches respectively, are presented in Table 1.

A series of titration experiments were performed on Probe **1** (aqueous phosphate buffer, pH 7) in order to determine the effect of DNA binding on the anthracene emission signal. As depicted in Fig. 1, the addition of aliquots of the cognate target **FM1** to a 1 μ M aqueous solution of Probe **1a**† resulted in a significant decrease in anthracene emission intensity, with quenching reaching approximately 85% once one equivalent of target was added. No further decrease was observed in the presence of excess amounts of target, which indicated the formation of a 1 : 1 complex, consistent with DNA duplex formation.

As expected, a titration with a random mis-matching strand **Z** resulted in no significant change in fluorescence emission. However, in contrast to the fully matching system, studies with targets **1MM** and **2MM**, containing one and two mis-matching bases respectively, resulted in an increase in emission intensity (Fig. 2). Once again, no additional changes were observed once more than one equivalent of each target was added to the probe.

DNA melting studies† confirmed that duplexes between Probe **1a** and each of the three targets form at the temperature used for the fluorescence studies. The T_m values of these three duplexes, as well as that for **FM1** and its complementary strand§ **FM2**, are presented in Table 2.

In order to explain these results, it is probably significant to note that the stability of the duplex **FM1**–Probe **1a**, where anthracene luminescence is quenched, is very similar to the stability of the duplex **FM1**–**FM2**. It is well documented that anthracene can intercalate into DNA⁸ and such an interaction not only increases duplex stability, as evidenced by higher T_m values, but also causes

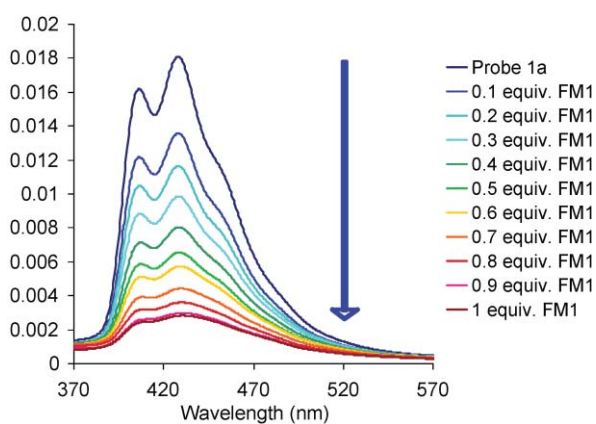


Fig. 1 Overlaid fluorescence spectra for the titration of up to 1 equiv. **FM1** with 1 μ M Probe **1a** (0.01 M phosphate, 0.2 M NaCl, pH 7.0 aqueous buffer). $\lambda_{\text{ex}} = 350$ nm.

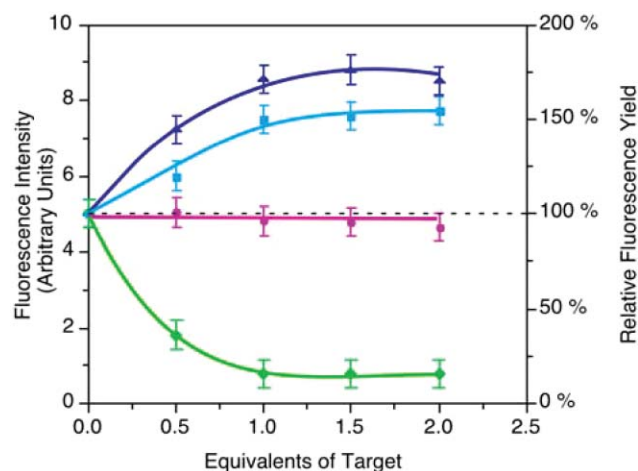


Fig. 2 Fluorescence titration plots at ca. 298 K for Probe **1a** with 0.5, 1.0, 1.5 and 2.0 equivalents of targets **FM1** (green), **1MM** (light blue), **2MM** (dark blue) and **Z** (pink) (0.01 M phosphate, 0.2 M NaCl, pH 7.0 aqueous buffer). $\lambda_{\text{ex}} = 350$ nm.

Table 2 T_m values for duplexes (pH 7.0 buffer, error margin ± 1 °C)

Duplex	T_m /°C	Change/°C
FM1 – FM2	52.0	—
FM1 –Probe 1a	50.0	–2
1MM –Probe 1a	39.5	–12.5
2MM –Probe 1a	36.0	–16

luminescence quenching, especially in GC rich regions.^{8a,8b} Anthracene intercalation within the **FM1**–Probe **1a** duplex would bring it into stacking contact with at least one GC unit. This would explain the fluorescence changes and also account for there being only a slight change in duplex stability when a stacking adenine base in **FM2** is replaced with a non-nucleosidic linker in Probe **1a**.

On the other hand, it is less clear why duplex formation between Probe **1a** and either **1MM** or **2MM** leads to an increase in emission intensity. Similar behavior has been observed when anthracene intercalates in AT vs. GC-rich regions of DNA,^{8a} but it is unlikely that the tether in **Probe 1a** is sufficiently long to allow the anthracene to bind distal to its point of attachment. Also, the T_m values clearly indicate that a large decrease in duplex stability results from the introduction of one and two base-pair mismatches, suggesting that for these duplexes, additional stabilisation from anthracene intercalation is not present. Since anthracene is only a weak intercalative DNA binder ($K \approx 150$ M⁻¹),^{8d} it is possible that the presence of a mismatch favours the anthracene being placed in a hydrophobic pocket, which limits quenching by stacking base residues and the aqueous environment.

In conclusion, we have developed a novel anthracene-tagged DNA probe that can detect a single base-pair mismatch in a target sequence which is of relevance to the development of novel SNP sensors. The unusual selectivity of the sensing signal (emission either increasing or decreasing depending on the sequence) stems from the local environment around the anthracene changing in response to duplex formation, rather than it relying on the thermodynamics of duplex formation *per se*.^{2a} Studies with different mismatching base sequences using a range of anthracene tags are now planned in order to rationalise these findings further.

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Notes and references

‡ The other diastereoisomer of Probe **1** also appears to be capable of SNP detection. Results will be reported elsewhere in due course.

§ To enable a comparison between the stability of different duplexes formed with **FM1**, the non-nucleosidic base in Probe **1** was changed for a mismatching adenosine in **FM2** as an isosteric replacement.

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