A New Homogeneous Identification Method for DNA

John Coates,[a] Peter G. Sammes,*[a] Gokhan Yahioglu,[a] Richard M. West[a] and Andrew J. Garman[b]

[a] *Molecular Probes Unit, Department of Chemistry, Brunel University, Uxbridge, Middlesex, UK UB8 3PH*
[b] *Zeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire, UK SK10 4TG*

A homogeneous method for identifying the presence of single-strand DNA targets is described which employs a cooperative sensitisation of Eu^{III} luminescence.

Rapid and precise registration of DNA sequences is of importance in the characterisation of genetic material, in particular to recognise sources of material and the presence or absence of mutations. Most available methods use heterogeneous methods involving the labelling of a complementary probe and separating the resultant hybridised duplex from the excess of the probe.[1] Herein we report a new approach to the direct *in vitro* (homogeneous) identification of target DNA strands[2] thus opening the possibility of high-throughput screens. The method also utilises time-resolved luminescence that offers higher sensitivities than normal fluorescence methods.[3]

The method is illustrated in Fig. 1. The probe is linked to a chelator to which is strongly coordinated a europium ion. The probe is then hybridised to the target DNA to form a double-stranded segment of DNA. In this situation neither the probe nor its duplex with the target gives a signal under irradiation with light. The europium ion has a very weak absorption coefficient since the excitation process is formally forbidden.[4]

Excitation of Eu^{3+} can be achieved by the presence of a sensitizer.[5] We have shown that, under certain conditions,[6] chelates of europium can accept a second ligand, such as a 1,10-phenanthroline-2,9-dicarboxylic acid, which acts both as a sensitizer and shielding agent to displace solvated water molecules; solvated water inhibits europium luminescence.[3] By choosing different combinations of chelate and sensitizing ligand one can select a range of binding constants of the second component. In our scheme (Fig. 1) the binding constant of the sensitizing ligand for the chelated europium ion is in the range K_{ass} 10^6–10^7 and thus, at concentrations of the two components at $<10^{-7}$ mol l^{-1}, little association between the reagents occurs. As a consequence the background signal for europium luminescence is also small and this is expected to fall away rapidly upon dilution.

Enhancement of the signal for europium luminescence may be achieved in several ways. We have initially chosen to use intercalation using a positively charged aromatic species such as the phenanthridinium group linked to the sensitizer. The binding constant of such species for intercalation,[7] into double-stranded DNA, is in the order of K_{ass} 10^6. Intercalation thus increases the local concentration of the sensitiser and this works in cooperation with the binding of the sensitiser to the metal ion. As a consequence the overall binding constant of the sensitiser to the metal is increased and, when a target is bound, results in the enhancement of the europium signal. Since in the presence of the target the signal is generated in a cooperative, intramolecular manner, the relative strength of the signal is expected to fall away less rapidly upon dilution.

For illustration, the chelating probe was prepared from a strand of DNA to which was attached, at the 5′-end, an aminohexyl group. Reaction of this with the anhydride of ethylenediaminetetraacetic acid at pH 9.0, followed by saturation with europium chloride gave the chelate derivative **1**. The chelate can be adequately purified by passing through a short Sephadex column (Pharmacia, NAP 5) to remove the excess of the reagents and lower molecular mass species. The target was the complementary DNA strand **2** and the sensitizer/intercalator was the phenanthroline dicarboxylic acid derivative **3**.

The probe DNA derivative **1** and the sensitizer **3**, as a 1 : 1 molar mixture (200 μl, was added to the target DNA, using a

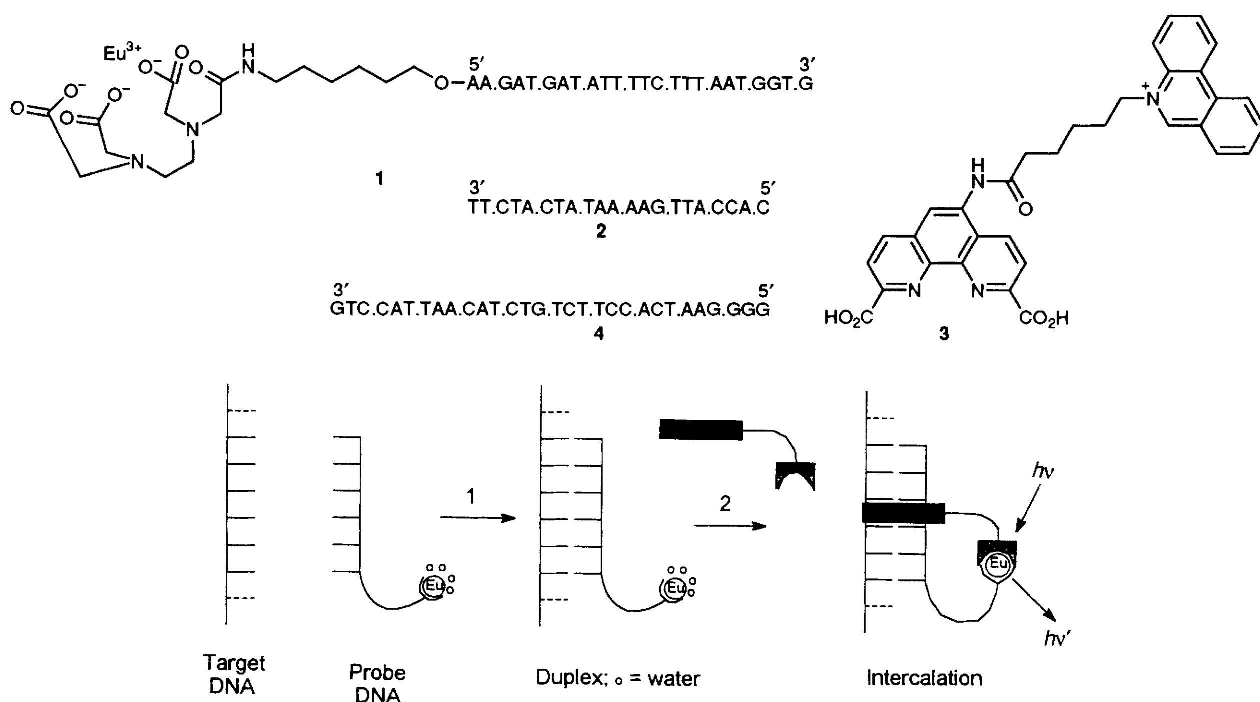

Fig. 1 Step 1: Hybridisation of probe with target. Step 2: Intercalator hunts to find site where binding to the europium ion can occur, to form stable, active complex.

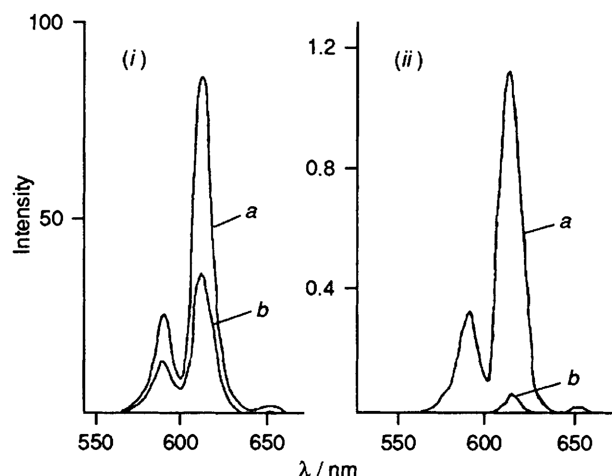

Fig. 2 Emission in range 550–650 nm observed on mixing reagents **1** and **3** with, (*a*) target **2** (match) and (*b*) target **4** (mismatch). Buffer as in text; (*i*) 5×10^{-7} mol l^{-1} **1**, 5×10^{-7} mol l^{-1} **3** and 5×10^{-7} mol l^{-1} target; (*ii*) × 56 dilution. Measurements on Perkin-Elmer LS50B instrument with delay time 0.1 ms, slit widths 10 nm.

hybridising solution of 25 μl Denhardt's solution, 600 μl buffer (10 mmol l^{-1} Tween 20, 1 mol l^{-1} NaCl, 10 mmol l^{-1} $MgCl_2$, 10 mmol l^{-1} HEPES). Final concentrations of the probe and sensitizer were 5×10^{-7} mol l^{-1}. The solution was hybridised at 42 °C before cooling to ambient temperature and reading the luminescence emission of the sensitised Eu^{3+} of the solution, using λ_{exc} 290 nm. As a control, in a separate experiment, the unrelated target DNA **4** was utilised. The results are illustrated in Fig. 2. As predicted, in the presence of the matching target **2** a stronger signal is observed compared to that found with the mismatch target **4**. Also, upon dilution, a more rapid decrease in the intermolecular (background) signal is observed than for the cooperative (intramolecular) signal obtained in the presence of the matching target.

We are currently extending this assay to the use with chromosomally derived DNA.

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