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DUAL-PROBE SYSTEM USING PYRENYLMETHYL-MODIFIED AMINO-LNA FOR MISMATCH DETECTION

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□ A dual-probe containing pyrenylmethyl amino-LNA has been developed for sensitive mismatch detection. While hybridization with complementary DNA/RNA results in very strong excimer signals, exposure to singly mismatched DNA/RNA targets results in significantly decreased excimer emission.

Keywords Single nucleotide polymorphism; pyrene; 2'-amino-LNA; oligonucleotides

INTRODUCTION

Single nucleotide polymorphisms (SNPs) are the most common form of mutations in the human genome and have been linked to many diseases. Thus, many SNP typing methods have been developed, among which fluorophore-labeled oligonucleotides in general, and dual-probes in particular (Figure 1), [1–5] play an important role.

RESULTS AND DISCUSSION

A pair of oligonucleotides containing 2'-*N*-pyren-1-yl-methyl-2'-amino-LNA $\mathbf{T}^{L}_{py}^{-1}$ **ON1**: 5'-d($\mathbf{C}\mathbf{A}^{L}\mathbf{C}$ $\mathbf{C}\mathbf{A}^{L}\mathbf{A}$ $\mathbf{C}\mathbf{T}^{L}_{py}$)-3' and **ON2**: 5'-d($\mathbf{T}^{L}_{py}\mathbf{C}\mathbf{T}$ $\mathbf{T}^{L}\mathbf{C}^{Me}\mathbf{C}^{L}$ $\mathbf{A}\mathbf{C}^{L}\mathbf{A}$)-3' were evaluated as dual-probes for detection of fully complementary nucleic acid targets. A high content of LNA monomers allowed the use of very short probes, there by likely leading to high mismatch discrimination. A pyrene excimer band is formed ($\lambda_{em}=480$ nm)

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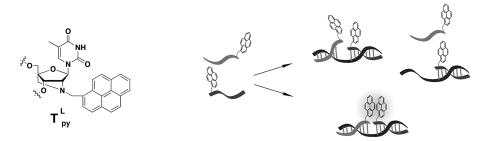


FIGURE 1 Left: Pyrene-functionalized 2′-amino-LNA monomer used in this study. Right: Principle of dual-probes.

upon hybridization of the dual-probes with complementary DNA/RNA (Figures 2a and b). A large decrease in excimer intensity is observed when the dual-probes are targeted toward DNA/RNA targets containing a single basepair mismatch, even if the fluorescence measurements are performed at temperatures well below the thermal denaturation temperatures of the

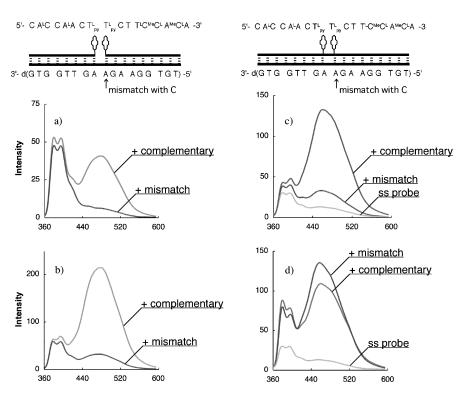


FIGURE 2 Signalling of full complementarity by fluorescence assay. a) dual-probe + DNA, b) dual-probe + RNA, c) covalently bonded probe + DNA, d) covalently bonded probe + RNA. Fluorescence measurements are carried out for the mixture of 0.15 μ M probe(s) and 0.11 μ M target DNA/ RNA in 10 mM phosphate buffer ([Na⁺] = 110 mM, [Cl⁻] = 100 mM, pH 7.0, at 19°C). \mathbf{A}^{L} , $^{Me}\mathbf{C}^{L}$ and \mathbf{T}^{L} denote LNA monomers.

ternary complexes. When a covalently bonded equivalent to the dual-probe system is targeted towards DNA/RNA complements, excimers are formed (Figures 2c and d). However, hybridization with mismatched targets only leads to a reduction in excimer intensity with DNA. Interestingly, the excimer intensity was slightly higher for the duplex between covalently bonded probe and mismatched RNA than for the corresponding duplex between the probe and complementary RNA. This could be due to a better overlapping between the pyrene units in the more flexible mismatched duplex.

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