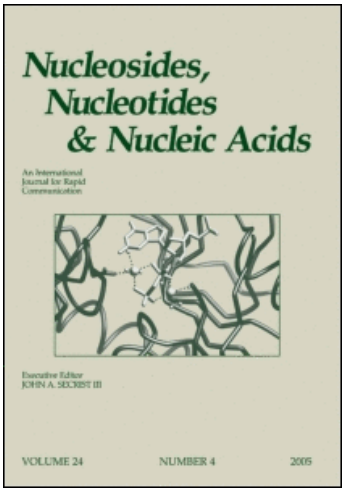

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Dual-Probe System Using Pyrenylmethyl-Modified Amino-Lna for Mismatch Detection

Tadashi Umemoto[a]; Patrick J. Hrdlicka[a]; B. Ravindra Babu[a]; Jesper Wengel[a]
[a] Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark, Odense, M, Denmark

To cite this Article Umemoto, Tadashi , Hrdlicka, Patrick J. , Babu, B. Ravindra and Wengel, Jesper(2007) 'Dual-Probe System Using Pyrenylmethyl-Modified Amino-Lna for Mismatch Detection', Nucleosides, Nucleotides and Nucleic Acids, 26: 10, 1261 — 1263
To link to this Article: DOI: 10.1080/15257770701528255
URL: http://dx.doi.org/10.1080/15257770701528255

Nucleosides, Nucleotides, and Nucleic Acids, 26:1261–1263, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701528255

Taylor & Francis
Taylor & Francis Group

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

Tadashi Umemoto □ *Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark, Odense M, Denmark*

Patrick J. Hrdlicka □ *Department of Chemistry, University of Idaho, Moscow, Idaho, USA*

B. Ravindra Babu and Jesper Wengel □ *Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark, Odense M, Denmark*

□ *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(C**A**^{L}C C**A**^{L}A C**T**$^{L}{}_{py}$)-3′ and **ON2**: 5′-d(**T**$^{L}{}_{py}$CT **T**^{L}C^{Me}**C**^{L} A**C**^{L}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 (λ_{em} = 480 nm)

Address correspondence to Tadashi Umemoto, RIKEN, Frontier Research System, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan. E-mail: tumemoto@riken.jp

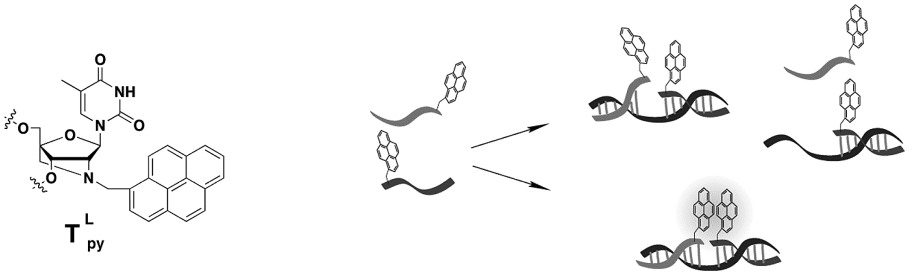

FIGURE 1 Left: Pyrene-functionalized 2′-amino-LNA monomer used in this study. Right: Principle of dual-probes.

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

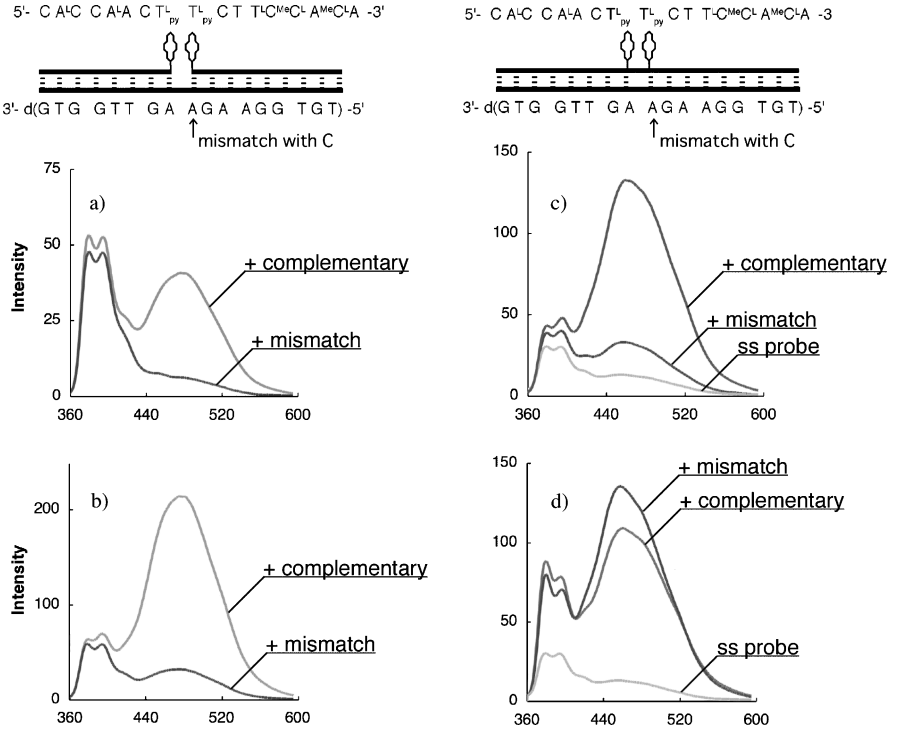

FIGURE 2 Signalling of full complementarity by fluorescence assay. a) dual-probe + DNA, b) dual-probe + RNA, c) covalently bonded probe + DNA, d) covalently bonded probe + RNA. Fluorescence measurements are carried out for the mixture of 0.15 μM probe(s) and 0.11 μM target DNA/ RNA in 10 mM phosphate 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.

REFERENCES

1. Hrdlicka, P.J.; Babu, B.R.; Sørensen, M.D.; Wengel J. Interstrand communication between 2′-*N*-(pyren-1-yl)methyl-2′-amino LNA monomers in nucleic acid duplexes: Directional control and signalling of full complementarity. *Chem. Comm.* **2004**, 1478–1479.
2. Masuko, M.; Ohtani, H.; Ebata, K.; Shimizu, A. Optimization of excimer-forming two-probe nucleic acid hybridization method with pyrene as a fluorophore. *Nucleic Acids Res.* **1998**, 26, 5409–5416.
3. Paris P.L.; Langenhan J.M.; Kool E.T. Probing DNA sequences in solution with a monomer–excimer fluorescence color change. *Nucleic Acids Res.* **1998**, 26, 3789–3793.
4. Dioubankova N.N.; Malakhov A.D.; Stetsenko D.A.; Korshun V.A. Detection of point mutations using pyrene. *Russian Chem. Bull. Int. Ed.* **2004**, 53, 463–470.
5. Bichenkova, E.V.; Savage, H.E.; Sardarian, A.R.; Douglas, K.T. Target-assembled tandem oligonucleotide systems based on exciplexes for detecting DNA mismatches and single nucleotide polymorphisms. *Biochem. Biophys. Res. Comm.* **2005**, 322, 956–964.