Khalij Libya Journal of Dental and Medical Research. 2025;9(2):268-274
https://doi.org/10.47705/kjdmr.259220
eISSN:2708-888X

Original article

Enhanced Sensitivity in Oligonucleotide Probes *via* Terminal Excimer Formation

Jamal Elbakay*, Shahrazad Eteer, Abdul Gbaj

Department of Medicinal Chemistry, Faculty of Pharmacy, University of Tripoli, Libya
Corresponding email. J.Elbakay@uot.edu.ly

Abstract
Fluorescence-based techniques are widely used to detect DNA mutations by monitoring changes in fluorescence signals that arise when DNA probes or dyes interact differently with mutated and normal DNA sequences. Such variations typically result from mismatched base pairing, altered melting temperatures, or changes in molecular interactions. This study aimed to investigate excimer fluorescence as a sensitive approach for distinguishing single-stranded and double-stranded DNA and for potential application in mutation detection. Excimer emission signals were analyzed in solution using fluorescence spectroscopy. Complementary oligonucleotides were labeled with pyrene fluorophores—one at the 5' end of the probe strand and the other at the 3' end of its complement. The proximity of the two pyrene groups upon hybridization was monitored to assess excimer formation. Hybridization of the complementary strands brought the two pyrene moieties into proximity, producing a strong excimer emission band at approximately 480 nm. This signal clearly differentiated double-stranded DNA from single-stranded forms. The system demonstrated high sensitivity to hybridization events and was suitable for monitoring DNA structural transitions such as denaturation and reassociation. Excimer fluorescence provides a reliable and sensitive method for detecting DNA hybridization and distinguishing between matched and mismatched sequences. The approach enables continuous monitoring of DNA interactions at low concentrations and offers promising potential for mutation and single-nucleotide polymorphism (SNP) detection in molecular diagnostics.
Keywords. *Terminally Located Probe Systems, Excimer, DNA Detection, Fluorescence, Stokes Shift.*

Received: *22/08/25*
Accepted: *30/10/25*
Published: *08/11/25*

Introduction

Reversible hybridization of complementary polynucleotides plays a fundamental role in the biological processes of replication, transcription, and translation. To understand these mechanisms at the molecular level, it is crucial to conduct detailed physical studies of nucleic acid hybridization. Such physical characterization also provides the basis for predicting nucleic acid behavior in vitro, particularly in hybridization assays designed to detect specific polynucleotide sequences [1].

Fluorescence measurements provide a more sensitive means of determining nucleic acid concentration compared with conventional solution-phase detection techniques. Moreover, the environmental sensitivity of fluorophores enables the distinction between hybridized and unhybridized nucleic acids without the need for physical separation methods. This principle was first demonstrated by attaching distinct fluorescent labels to the termini of oligonucleotides designed to hybridize to adjacent regions of a complementary DNA strand [2,3]. With appropriate fluorophore selection, hybridization of the dual-labeled strands to their complementary target produced a measurable fluorescence signal arising from interactions between the terminal labels. For example, split-probe systems based on excimer fluorescence were first described by Ebata *et al.* [4-6], who attached pyrene to the 5'-terminus of one oligonucleotide probe and to the 3'-terminus of the other oligonucleotide probe. The probes bound to adjacent regions of the target, bringing the pyrene molecules into proximity, forming an excimer. Excimer emission from oligonucleotides containing 5-(1-pyrenylethynyl)uracil [7], trans-stilbene [8], and perylene [9] has also been reported. A Study reported [10] that an emissive exciplex can be formed by the juxtaposition of two externally oriented exciplex-forming partners (pyrene and naphthalene) at the interface (nick region) of tandem oligonucleotides forming a duplex of some kind on hybridization with their complementary target strand [10]. As reported by Ishii *et al.* (2025), a peptide nucleic acid (PNA) twin probe, consisting of two PNAs each containing a fluorescent dye with pyrene at one end, detects target DNA sequence-specifically through pyrene excimer emission.

In this study, to advance the development of this probe system, we further investigated the fluorescence properties of the PNA twin probes P1 and P2, and found that excimer fluorescence was significantly reduced when a mismatched base in the DNA sequence was present at the site of P1 closest to the pyrene [11]. Our focus has been on using excimer fluorescence signals to investigate the hybridization of two fluorophore-labeled complementary DNA strands, as depicted in (Figure 1). Fluorescent labels, such as pyrene and pyrene, attached to the termini of these strands exhibited strong interactions upon

Khalij Libya Journal of Dental and Medical Research. 2025;9(2):268-274
https://doi.org/10.47705/kjdmr.259220
eISSN:2708-888X

hybridization, reflecting efficient excimer formation between specific fluorophore pairs. The primary aim of this study is to investigate the hybridization behavior of complementary DNA strands through excimer fluorescence analysis. Specifically, this work focuses on utilizing excimer fluorescence signals to monitor and characterize the hybridization process between two fluorophore-labeled complementary DNA strands and examining the efficiency of excimer formation between identical fluorophores (e.g., pyrene–pyrene pairs) attached at the termini of DNA strands upon duplex formation. Correlating fluorescence intensity and spectral shifts with the degree of hybridization, thereby establishing excimer emission as a sensitive optical indicator of DNA duplex formation and providing a physical basis for understanding DNA hybridization mechanisms that can support the design of fluorescence-based mutation or SNP detection assays. The specific objectives of this study were to design and synthesize complementary oligonucleotides labeled with pyrene fluorophores at opposite termini, analyze excimer emission behavior upon hybridization using fluorescence spectroscopy, evaluate the ability of excimer fluorescence to monitor DNA hybridization, denaturation, and reassociation processes, and assess the suitability of this method for mutation and single nucleotide polymorphism (SNP) detection.
Terminally located probe systems based on two anti-parallel strands were investigated in this paper using the base sequences shown in (Figure 1).

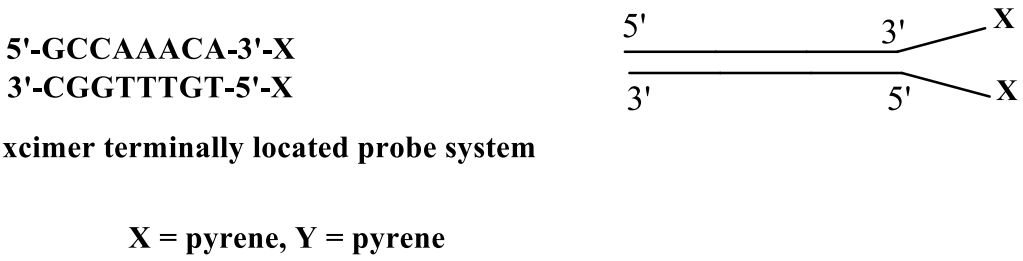

Figure 1. Base sequences of the terminally labeled probe systems used in this study.

Methods

The excimer constructs used standard DNA base/sugar structures in both complementary probes. The probes were a part of the *Leishmania major* chromosome 1 sequence (Genbank reference [AE001274 nucleotides 90014-90029]: cccttccctc cctcgattctg**tgtttggc**g aggacacccg cctcgacgac atcacacacg (the **bold** bases provided the complement to the probe used). The ExciProbe probes had the sequence 3'pACAAACCG-5' and 3' CGGTTTGT-5'-p. The probes were supplied with a free 3' or 5'-phosphate group (p).
Reagents of the highest quality available were purchased from the suppliers indicated. DNA probes and DNA targets from Proligo, Paris (Sigma- Proligo), deuterium oxide was from Cambridge Isotope Laboratories (Goss-Scientific Instruments Ltd, UK). All water used was distilled and purified by ion exchange and charcoal using a MilliQ system (Millipore Ltd, UK). Tris buffer was prepared from analytical reagent-grade materials. Values of pH were measured using a Hanna-instruments (Portugal)HI 9321 microprocessor pH meter, calibrated with standard buffers (Sigma-Aldrich) at 20°C.

HPLC

HPLC purifications of probes were performed on an Agilent 1100 Series HPLC system, consisting of a quaternary pump with solvent degasser, a diode-array module for multi-wavelength signal detection using an Agilent 1100 Series UV-visible detector, and an Agilent 1100 Series fluorescence detector for online acquisition of excitation/ emission spectra. The system had a manual injector and a thermostatted column compartment with two heat exchangers for solvent pre-heating. The HPLC system was operated through Agilent HPLC 2D ChemStation Software. Depending on the purification performed, the columns used were: Zorbax Eclipse X DB-C8 column (length 25 cm, inner diameter 4.6 mm, particle size 5µm), or a Luna C18 (2) column (length 25 cm, inner diameter 4.6 mm, particle size 5 µm) with elution using an increasing gradient (0–50%) of acetonitrile in water (fraction detection at 260, 280, and 340 nm).

UV-visible spectrophotometry

UV–visible absorption spectra were measured at 20°C on a Cary-Varian 1E UV–visible spectrophotometer with a Peltier-thermostatted cuvette holder and Cary 1E operating system/2 (version 3) and CARY1 software. Quantification of the oligonucleotide components used millimolar extinction coefficients (ε_{260}) of 79.9 for ExciProbe-3'-phosphate, 70.2 for ExciProbe-5'-phosphate. The extinction coefficients were

Khalij Libya Journal of Dental and Medical Research. 2025;9(2):268-274
https://doi.org/10.47705/kjdmr.259220
eISSN:2708-888X

calculated by the nearest neighbour method [12] and the contribution of the exci-partners (two molecular species that come together to form the excimer complex and not pyrene -pyrene) was neglected.

Melting Temperature Studies

Optical melting curves of the complexes were recorded using a Varian Cary 1E UV–visible spectrophotometer with a 1 mL quartz cuvette (pathlength 1.0 cm). Thermal denaturation (T_m) measurements, monitored at 260 nm with an accuracy of ±0.1 °C, were performed for 1:1 complex at 2.5 µM component concentrations in 60% TFE/Tris buffer (10 mM Tris, pH 8.4, 100 mM NaCl). Complementary melting data were obtained from fluorescence emission spectra: samples were heated to 80 °C at 0.25 °C min^{-1}, using excitation/emission wavelengths of 340/376 nm for the pyrene monomer and 350/480 nm for the excimer. Spectra were recorded at 0.5 °C intervals. Samples were subsequently cooled at 0.13 °C min^{-1}, and emission spectra were recorded again. T_m values were determined from the melting curves obtained during the cooling cycle.

Spectrophotofluorimetry

Fluorescence emission and excitation spectra were recorded in 4-sided quartz thermostatted cuvettes using a Peltier-controlled-temperature Cary-Eclipse spectrofluorophotometer. All experiments were carried out at 5°C. Hybridisation: Duplex formation was induced by sequential addition of 5'-probe and 3'-probe. The mole ratio of all oligonucleotides, 5'-probe and 3'-probe used was 1:1, the concentrations of each of the components were 2.5 µM. Tris buffer was also added at a concentration of 2.5 µM either with or without 60% TFE, and volume was made up to 1000 µL with water. Excitation wavelengths of 340 nm (for the pyrene monomer) and 350 nm (for the full terminally located probe systems) were used, at a slit width of 5 nm, and recorded in the range of 350-650 nm. Emission spectra were recorded after each sequential addition of each component to record the change in emission due to each addition. A baseline spectrum of buffer and water, or buffer, water, and 60% TFE, was always carried out first. On each addition, the solution was left to equilibrate for approximately 5 minutes in the fluorescence spectrophotometer, and emission spectra were recorded until no change in the spectra was seen to ensure it had been reached. Orders of addition for experiments using 5'-monopyrene were firstly, then the 3'-pyrene probe. Control experiments were conducted using first 5'-pyrene, followed by a 3'-free oligonucleotide probe. Most spectra were buffer and 3'-phosphate corrected.

Control experiments

Control experiments were carried out in 60% TFE/ Tris buffer, as for the experimental systems, using the standard method described above. To establish for the present system whether the apparent excimer emission is the result of such background effects or arises from the hypothesised excimer structures. Then fluorescence melting curve experiments (based on excitation 350 nm and emission 480 nm for the excimer) were performed using a Cary Eclipse fluorescence spectrophotometer by measuring the change in fluorescence intensity for the excimer with temperature. T_m was also determined spectrophotometrically by measuring the change in absorbance at 260 nm with temperature. Melting temperatures (T_m) were determined either by taking the point at half the curve height as T_m or by using the first derivative method.

Synthesis and oligonucleotide modification

Attachments of 1-pyrenemethylamine to oligonucleotide probes were as described [10,13].

Results

Excimer formation using terminally located probe systems

Fluorescence studies were made for solutions of 3'-pyrenyl and 5'-pyrenyl oligonucleotides with both probes complementary to each other (Figure 1).

Figure 2 shows excitation and emission spectra for (**A**) the 3'-pyrenyl oligonucleotide alone in 60% TFE/ Tris buffer (0.01 M Tris, 0.1 M NaCl, pH 8.4) at 5 °C, (**B**) 3'-pyrenyl probe hybridised to the 5'-pyrenyl oligonucleotide. On excitation at 350 nm, the 3'-pyrenyl probe showed fluorescence typical of pyrene light emission from a localized excited electronic state of a pyrene (LES emission, □max = 376, 395 nm). Addition of the 5'-pyrenyl probe resulted in immediate quenching of the LES emission at 395 nm to less than one-third of its original value and the appearance of a new, broad emission band (□$_{max}$ = 480 nm) characteristic of pyrene excimer fluorescence (when the full terminally located system had formed)[4,5,14]. Addition of the 5'-pyrenyl probe to the 3'-pyrenyl also caused a slight red shift in both excitation (from 342 nm to 349 nm) and emission (from 376 nm to 378 nm; λex 350 nm) spectra, consistent with duplex formation [4,5,14].

Khalij Libya Journal of Dental and Medical Research. 2025;9(2):268-274
https://doi.org/10.47705/kjdmr.259220
eISSN:2708-888X

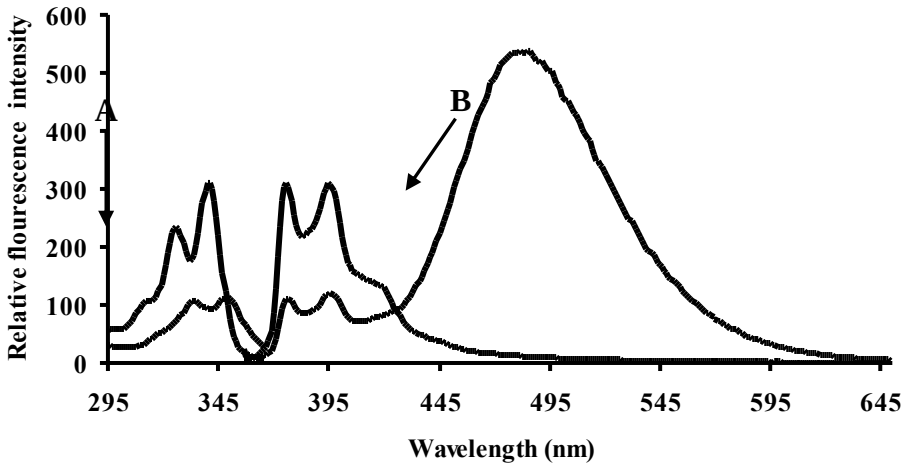

Figure 2. Excitation and emission spectra of the terminally located probesystem (A) 3'-pyrenyl probe alone and (B) 3'-pyrenyl+5'-pyrenyl probe in 60% TFE/0.01 M Tris, 0.1 M NaCl, pH 8.4) at 5 °C. Component concentrations were 2.5 µM (equimolar).

Control experiments for a terminally located excimer system

Control experiments on a 1:1 mixture of 3'-pyrenyl and 5'-free-phosphate oligonucleotides were carried out in 60% TFE/0.01 M Tris, 0.1 M NaCl, pH 8.4, to determine if the fluorescence was from pyrene interacting as an excimer with the intended pyrene exci-partner, or as an excimer by interaction with bases of the oligonucleotides. The sequence of the control system used is shown in (Figure 1).

The 3'-pyrenyl oligonucleotide probe showed no band at 480 nm in the absence of 5'-phosphate oligonucleotide (Figure 3). Addition of the 5'-phosphate oligonucleotide resulted in a slight shift in λ_{max} of LES emission to 379 nm, consistent with hybridisation of two complementary probes. However, no marked 480 nm band was seen, even after heating the system to 60 °C and re-annealing by slowly cooling back to 5 °C. The weak fluorescence emission at 480 nm for the control duplex (before and after heating and cooling, Figure 3) on duplex formation may be real and could be related to excimer formation, due to *intra*-molecular interaction of pyrene within the assembled duplex. However, relative to the full system with both 3'- and 5'-pyrenyl groups (Figure 3), the emission at 480 nm is insignificant. These findings therefore reinforce the conclusion that the excimer emission serves as a reliable indicator of hybridization between two pyrene-labeled strands and not a byproduct of duplex formation alone. The control system validates the specificity of the excimer signal, underscoring its suitability for use in sensitive fluorescence-based monitoring of DNA association and dissociation.

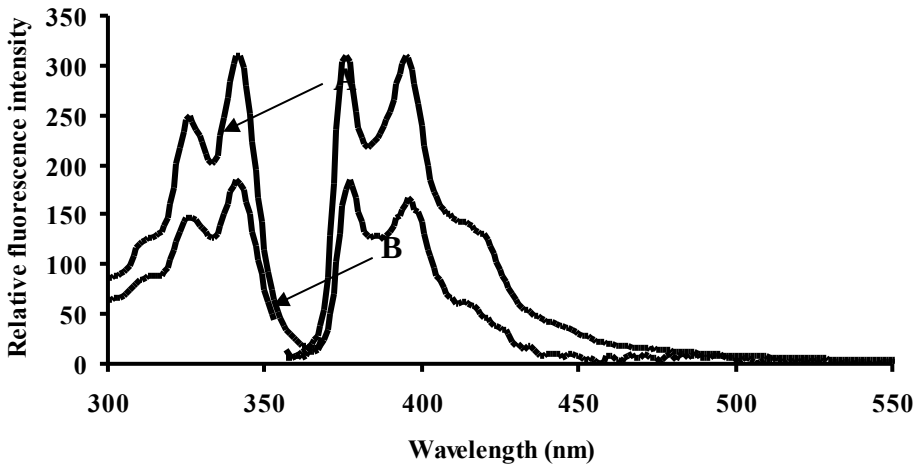

Figure 3. Emission spectra for control terminally located system (A) 3'-pyrenyl oligonucleotide and (B) 3'-pyrenyl oligonucleotide plus 5'-phosphate in 60% TFE/10 mM Tris, 0.1 M NaCl, pH 8.4 at 5 °C. Excitation wavelength 350 nm, slitwidth 5 nm. Equimolar component concentration was 2.5 µM.

Khalij Libya Journal of Dental and Medical Research. 2025;9(2):268-274
https://doi.org/10.47705/kjdmr.259220
eISSN:2708-888X

Melting Temperature (T_m)

Thermal denaturation profiles for the fluorophore-labeled complementary and mismatched DNA duplexes were analyzed using both UV absorbance and fluorescence emission. The melting temperature (T_m) was determined as the midpoint of the transition curve obtained from the first derivative of absorbance (260 nm) and fluorescence (excimer 480 nm) data during the cooling phase. The melting temperature (T_m) for the two perfectly complementary probes (Probe A and Probe B as ExciProbe) was determined by both UV absorbance and fluorescence methods. Using UV detection at 260 nm, the duplex exhibited a T_m = 24.1 ± 0.1 °C, showing a sharp and cooperative transition indicative of high duplex stability. Fluorescence measurements monitored at the pyrene excimer emission (480 nm) yielded a comparable T_m = 24.2 ± 0.1 °C, accompanied by a strong excimer signal, confirming efficient stacking interactions between the terminal pyrene fluorophores upon hybridization.

Discussion

Morrison *et al.* showed that attaching fluorescent labels to the respective 3'- and 5'-termini of complementary DNA strands gave strong quenching interactions between particular fluorophore pairs upon hybridisation [15,16]. Thus, terminally located probe duplex structures of short oligonucleotides, similar in length to those used in this paper, can form stable duplexes. Several groups have incorporated excimer-forming molecules such as stilbene into oligonucleotides. Lettsinger*et al.* incorporated a stilbene-modified nucleotide at the 5'-end of one oligonucleotide and a stilbene-modified nucleotide at the 3'-end of the other [17,18]. By hybridising the two probes to each other, the stilbene moieties came into proximity, and an excimer band at 455 nm was generated. Yamana *et al.* used a terminal position system that incorporated a pyrene-modified nucleotide at the 5'-end of one probe and a pyrene-modified nucleotide at the 3'-end of the other [19]. By hybridising the two probes to each other in phosphate buffer (pH 7.0) containing 0.1 M NaCl, the pyrene moieties came into proximity, and an excimer band at 480 nm was generated. Figure 2 shows fluorescence typical of pyrene LES emission (λ_{max} = 376, 395 nm) for a 3'-pyrenyl labelled oligonucleotide alone. In 10 mM phosphate buffer (pH 7.0), 20 % v/v DMF, 0.2 M NaCl at 25 °C, a literature system gave λ_{max} = 377, 396 nm [4,5,14]. Addition of the 5'-pyrenyl probe resulted in immediate quenching of the LES emission at 395 nm to less than one-third of its original value and the appearance of a new, broad emission band (λ_{max} = 480 nm) characteristic of pyrene excimer fluorescence (Figure 2).

Melting experiments provide further strong evidence of duplex formation. The terminally located probe systems showed sigmoid single-transition melting curves spectrophotometrically (A260 or A350) or spectrofluorometrically from fluorescence intensity at 340 nm for the LES (λex) and 376 nm (λem) for pyrene monomer and at 350 nm for the LES (λex) and 480 nm (λem) for the excimer. Additional evidence of duplex formation comes from the emission spectra, as one probe oligonucleotide alone did not give an excimer signal in the absence of the other complementary probe.

Additional evidence of duplex formation and reversibility came from experiments involving a heating and cooling cycle. Experiments with terminally located probe systems at different temperatures revealed that the excimer intensity decreased as the temperature increased and eventually disappeared. This process is reversible, providing further evidence of duplex formation. A better-formed duplex structure probably enables the exci-partners to be better positioned for excimer formation. The reappearance of the excimer spectra on re-cooling indicates that no destruction of the components occurs on heating the system.

The red-shifted structureless band at ~ 480 nm is characteristic of excimer or emission, but could be due to interaction of the excimer partners with each other or nucleobases, as pyrene can form an excimer with certain nucleotide bases, especially guanine and to a lesser extent thymidine [20,21]. Also, some oligonucleotide sequences show weak exciplex emission from pyrene attached to their 5'-termini in the absence of any added (complementary) oligonucleotide [22]. Thus, it is important to establish for the terminally located system the origin of the emission at 480 nm.

Usually, emission typical of the pyrene LES was observed in the absence of the 3'-phosphate probe, indicating little or no background excimer emission due to pyrene interacting with the probe oligonucleotide. On addition of the 3'-pyrene probe, excimer emission was detected at 5 °C, accompanied by the red shift and quenching of LES emission. Heating the system caused the excimer emission intensity to decrease due to dissociation of the duplex structure. On re-cooling the system, excimer emission reappeared. The Tm values by fluorescence and UV-visible methods were similar and of the magnitude expected for such a system (8-mer duplex) [17].

Experiments performed with a 5'-pyrene-labeled probe and one 3'-unlabeled probe, bearing only a phosphate group at its terminus, showed no excimer emission (Figure 3). This suggests that the second excimer partner is required for excimer emission, rather than pyrene interacting with the nucleotide bases. The thermal denaturation profiles obtained from UV absorbance at 260 nm and fluorescence emission at 480 nm demonstrate that our fluorophore-labeled complementary probes (Probe A/Probe B) form a stable

Khalij Libya Journal of Dental and Medical Research. 2025;9(2):268-274
https://doi.org/10.47705/kjdmr.259220
eISSN:2708-888X

duplex (T_m ≈ 24.1 ± 0.1 °C by UV; 24.2 ± 0.1 °C by excimer fluorescence). The sharp transition and the strong excimer signal indicate efficient hybridization and effective stacking interactions between the terminal pyrene labels [19]. These findings align with prior studies showing that pyrenelabelled oligonucleotides can report duplex formation by excimer emission. For example, it has been demonstrated that a pyrene–excimer–forming probe system could detect single-base mismatches, noting that excimer emission was significantly reduced when duplex formation was compromised. Similarly, Znosko and colleagues (in the study of bispyrenelabelled oligonucleotides) found that excimer fluorescence intensity correlated with duplex stability: higher excimer/monomer ratios corresponded to higher T_m values [23]. In our case, the near-identical T_m values from UV and excimer fluorescence confirm that the excimer signal is a reliable proxy for duplex thermal stability. The pyrene labels likely promote stacking interactions at the termini, which enhance duplex stability under our conditions. The literature supports the idea that such hydrophobic fluorophore stacking can modestly increase T_m (as seen in the bispyrene study where ΔT_m was +2 to +3 °C compared to unmodified duplexes) [19].

Furthermore, our results highlight the potential of excimer-based fluorescence monitoring in hybridization assays: because the formation of the excimer requires proximity of the fluorophores (which itself requires proper duplex formation), a strong excimer emission serves as a molecular indicator that hybridization is complete and the duplex is thermally stable. This is particularly beneficial for applications such as SNP/mutation detection, where destabilization (manifested as a lowered T_m and diminished excimer emission) signals the presence of a mismatch [24]. In summary, our findings are consistent with the established literature and demonstrate that excimer fluorescence not only tracks hybridization but also reflects duplex thermodynamics. The strong correlation between UV-derived T_m and excimer-derived T_m in our study underscores the utility of this method in biophysical characterization of nucleic acid hybridization.

The results obtained in this paper evaluate the first case of an oligonucleotide. The clear correlation between excimer emission and DNA duplex formation highlights the potential of terminally pyrene-labeled oligonucleotides as powerful fluorescence-based probes for monitoring hybridization events. Because excimer formation depends on the precise spatial arrangement of the pyrene moieties, the signal provides direct structural information on molecular association and dissociation in real time. This property can be exploited in the design of sensitive nucleic acid biosensors capable of detecting complementary sequences at low concentrations without the need for separation or washing steps. Moreover, the reversible nature of the excimer signal allows for repeated monitoring of thermal denaturation and reassociation processes, offering a convenient tool for studying the thermodynamic and kinetic behavior of short DNA duplexes. These results demonstrate that pyrene excimer fluorescence is not only a diagnostic marker of duplex formation but also a versatile platform for the development of hybridization-based analytical and diagnostic assays.

Conclusion

This study demonstrates that terminally pyrene-labeled complementary oligonucleotides can form stable duplexes capable of producing strong excimer fluorescence upon hybridization. The appearance of a distinct red-shifted emission band at approximately 480 nm, together with consistent thermal denaturation profiles obtained by both UV absorbance and fluorescence spectroscopy, confirms that excimer emission serves as a reliable optical indicator of duplex formation and stability. The novelty of this approach lies in its ability to directly correlate excimer intensity with hybridization state, enabling real-time, label-specific monitoring of DNA structural transitions without the need for complex instrumentation or additional reagents. This system provides a sensitive and reversible means to study DNA denaturation, reassociation, and mismatch discrimination, making it particularly attractive for mutation and SNP detection assays. Future work should explore optimization of probe design, sequence length, and fluorophore positioning to improve sensitivity toward single-base mismatches and to expand the applicability of this excimer-based platform in biosensing and molecular diagnostic technologies.

Conflict of interest. Nil

References

1. Balakin KV, Korshun VA, Mikhalev II, Maleev GV, Malakhov AD, Prokhorenko IA, et al. Biosensors & Bioelectronics. 1999;14(6):597.
2. Bichenkova EV, Gbaj A, Walsh L, Savage HE, Rogert C, Sardarian AR, et al. Org Biomol Chem. 2007;5(7):1039-51.
3. Bichenkova EV, Savage HE, Sardarian AR, Douglas KT. Detection of peptide nucleic acids by fluorescent intercalator displacement: a preliminary study. Biochem Biophys Res Commun. 2005;332(4):956-64.
4. Cantor CR, Warshaw MM, Shapiro H. Oligonucleotide interactions. III. Circular dichroism studies of the conformation of deoxyoligonucleotides. Biopolymers. 1970;9(9):1059-77.

https://doi.org/10.47705/kjdmr.259220

eISSN:*2708-888X*

5. Ebata K, Masuko M, Ohtani H, Jibu M. Fluorescence characteristics of pyrene derivatives attached to the terminal of oligonucleotides. Nucl Acids Symp Ser. 1995;(34):187-8.
6. Ebata K, Masuko M, Ohtani H, Kashiwasake-Jibu M. Photochem Photobiol. 1995;62(5):836-9.
7. Geacintov NE, Moller W, Zager E. Energy transfer and fluorescence quenching in complexes between oligonucleotides and acridine orange. Biol Mol Proc Int Conf. 1976:199-206.
8. Hays A, Wissel M, Colletti K, Soon R, Azadeh M, Smith J, et al. AAPS J. 2024;26(1):24.
9. Ishii K, Shigeto H, Yamamura S, Imai Y, Ohtsuki T, Kitamatsu M. Org Biomol Chem. 2025;23(36):6557-63.
10. Kostenko E, Dobrikov M, Pyshnyi D, Petyuk V, Komarova N, Vlassov V, et al. Nucleic Acids Res. 2001;29(17):3611-20.
11. Letsinger RL, Wu T. Use of a stilbenedicarboxamide bridge in stabilizing, monitoring, and photochemically altering folded conformations of oligonucleotides. J Am Chem Soc. 1995;117(28):7323-8.
12. Letsinger RL, Wu T. Controls of nucleoside conformation and reactivity by a steric interaction between sugar and base. J Am Chem Soc. 1994;116(3):811-2.
13. Lewis FD, Wu T, Burch EL, Bassani DM, Yang JS, Schneider S, et al. J Am Chem Soc. 1995;117(34):8785-92.
14. López-Tena M, Winssinger N. Nucleic acid templated chemistry for the synthesis of functionalized peptides and glycopeptides. Org Biomol Chem. 2024;22(29):5759-67.
15. Manoharan M, Tivel K, Zhao M, Nafisi K, Netzel TL. J Phys Chem. 1995;99(47):17461-72.
16. Masuko M, Ebata K, Ohtani H. Nucl Acids Symp Ser. 1996;(35):165-6.
17. Masuko M, Ohtani H, Ebata K, Shimadzu A. Optimization of excimer-forming two-probe nucleic acid hybridization method with pyrene as a fluorophore. Nucl Acids Res. 1998;26(23):5409-16.
18. Morrison LE, Halder TC, Stols LM. Solution-phase detection of polynucleotides using interacting fluorescent labels and competitive hybridization. Anal Biochem. 1989;183(2):231-44.
19. Morrison LE, Stols LM. Sensitive fluorescence-based thermodynamic and kinetic measurements of DNA hybridization in solution. Biochemistry. 1993;32(12):3095-104.
20. Nath P, Mahtaba KR, Ray A. Sensors (Basel). 2023;23(3).
21. Savage H. Fluorescent intercalator displacement (FID) assays for the rapid screening of nucleic acids and ligands [PhD thesis]. Manchester (UK): The University of Manchester; 2003.
22. Shimadzu A, Ohtani H, Ohuchi S, Sode K, Masuko M. Nucl Acids Symp Ser. 1998;(39):45-6.
23. Thiede J, Rothenbühler S, Iacovache I, Langenegger SM, Zuber B, Häner R. Org Biomol Chem. 2023;21(37):7908-12.
24. Yamana K, Nunota K, Nakano H, Sangen O. Tet Lett. 1994;35(17):2555-8.