Talanta 88 (2012) 593–598

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

FRET detection of DNA sequence via electrostatic interaction of polycationic phenyleneethynylene dendrimer with DNA/PNA hybrid

Paitoon Rashatasakhon[a], Kunnigar Vongnam[b], Warathip Siripornnoppakhun[a], Tirayut Vilaivan[a], Mongkol Sukwattanasinitt[a,*]

[a] Center for Petroleum, Petrochemicals and Advanced Materials, Department of Chemistry, Faculty of Science, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand
[b] Program of Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330, Thailand

ARTICLE INFO

Article history:
Received 23 October 2011
Received in revised form
13 November 2011
Accepted 14 November 2011
Available online 19 November 2011

Keywords:
DNA
Dendrimer
Fluorescence sensor
FRET
Peptide nucleic acid

ABSTRACT

Interaction between a polycationic compound and DNA is a useful phenomenon for development of a new DNA sensing system. In this work, dendritic polycationic phenyleneethynylene fluorophores are investigated as a Förster resonance energy transfer (FRET) donor for the detection of DNA hybridization in conjunction with a fluorescein-labeled pyrrolidinyl peptide nucleic acid (Fl-acpcPNA) probe. The first generation dendrimer is an efficient energy donor for the fluorescein acceptor but also shows non-specific FRET signal with Fl-acpcPNA. The addition of *N*-methyl 2-pyrrolidone can virtually completely remove the non-specific interaction between Fl-acpcPNA and the dendrimer. Under the optimal condition, the complementary DNA gives a distinctively high FRET ratio (1.42) comparing with those of the non-complementary (0.26) and singly mismatched (0.51) DNAs. The FRET ratio responses linearly with the DNA concentration with the detection limit lower than 1 nM. The FRET ratio is even higher for the complementary target DNAs with extra hanging nucleotide sequences, which is a more frequently encountered scenario in real applications.

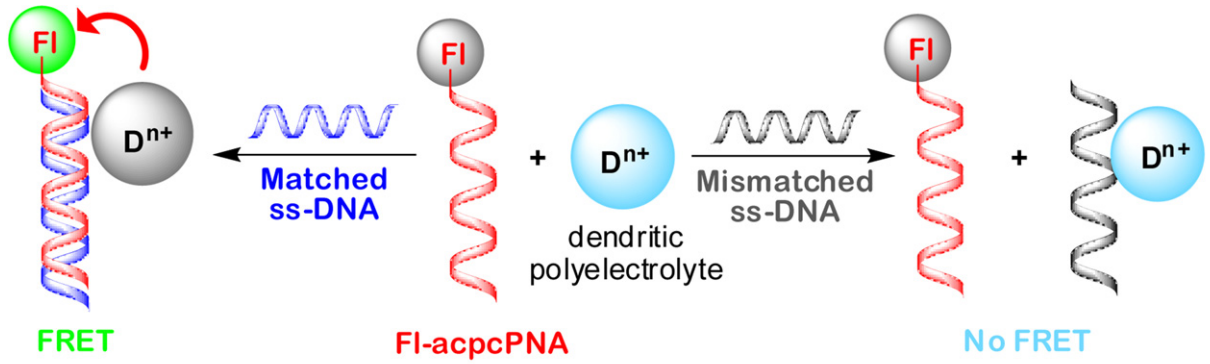
1. Introduction

Detection of DNA sequences is indispensable in medical diagnostics and biochemical analyses. During the past decades, we have witnessed several major developments of various techniques for the detection of DNA sequences based on DNA hybridization process including fluorimetry [1–3], colorimetry [4,5], electrochemistry [6–8], and surface plasmon resonance [9,10]. Homogeneous fluorescence analysis in solution is considered as one of the most convenient and sensitive methods. Direct use of fluorescent dyes which are capable of intercalating or binding into the grooves of double-stranded DNA (dsDNA) with concomitant fluorescence enhancement, such as ethidium bromide, SYBR Green® and PicoGreen®, as reporters for DNA hybridization is probably the most simple and common practice. Enhanced fluorescent signals by mismatched hybridized or even unhybridized single-stranded DNAs (ssDNA) are also often encountered due to their significant binding to these dyes [11,12]. To increase specificity of the analytical method, Förster resonance energy transfer (FRET) between energy donor and acceptor fluorophores has been successfully exploited [13,14]. One of the most intriguing developments involves the use of a polycationic π-conjugated polymer as a FRET donor and a flurophore-labeled ssDNA as a FRET acceptor [15,16]. Fluorescein-labeled peptide nucleic acid (Fl-PNA), a neutral peptide analog of DNA, has also been applied in a FRET-based detection of specific DNA sequences via PNA/DNA Watson–Crick hybridization [17–19]. The successful use of PNA probe has been attributed to two main reasons. First, PNA/DNA hybrid electrostatically interacts with the polycationic FRET donor more strongly, and second, the neutral unhybridized PNA and the PNA/DNA duplex is more readily and specifically formed than the analogous DNA/DNA duplex [20–23]. As a result, the use of PNA in place of DNA significantly increased the specificity such that even a single mismatched base in the DNA target can be readily distinguished with a considerably lower FRET signal [19].

Comparing with small molecule, conjugate polymers containing multiple fluorogenic moieties offer advantages in signal amplification and large molecular optical cross section [24–26]. The use of polycationic conjugated polymers as FRET donors for the detection of PNA/DNA hybridization however have been limited to a class of linear poly(phenylene-co-fluorene). The linear conjugated polymers usually possess broad distribution of molecular weights which may be not readily reproducible from lab to lab or even batch to batch. Unpredictable secondary structures of linear polymeric chains in aqueous media also add subtle issues in the interpretation of its intermolecular interaction. We are interested in exploring

* Corresponding author. Tel.: +66 2 2187620; fax: +66 2 2187598.
E-mail address: smongkol@chula.ac.th (M. Sukwattanasinitt).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.11.041



Fl-acpcPNA: 5' (*N*) Fluorescein-linker-CTAAATTCAGA-Lys-NH_2 (*C*) 3'
linker = $NHCH_2CH_2OCH_2CH_2OCH_2CO$
cDNA (complementary DNA): 3'-GATTTAAGTCT-5'
ncDNA (non-complementary DNA): 3'-AGACTTAAATC-5'
smDNA (singly mismatched DNA): 3'-GATTCAAGTCT-5'
cDNA' (cDNA + 10 extra bases): 3'-ATTGAGATTTAAGTCTCTATA-5'
cDNA'' (cDNA + 20 extra bases): 3'-ATTGGTCCTTGATTTAAGTCTATCGTCTATA-5'

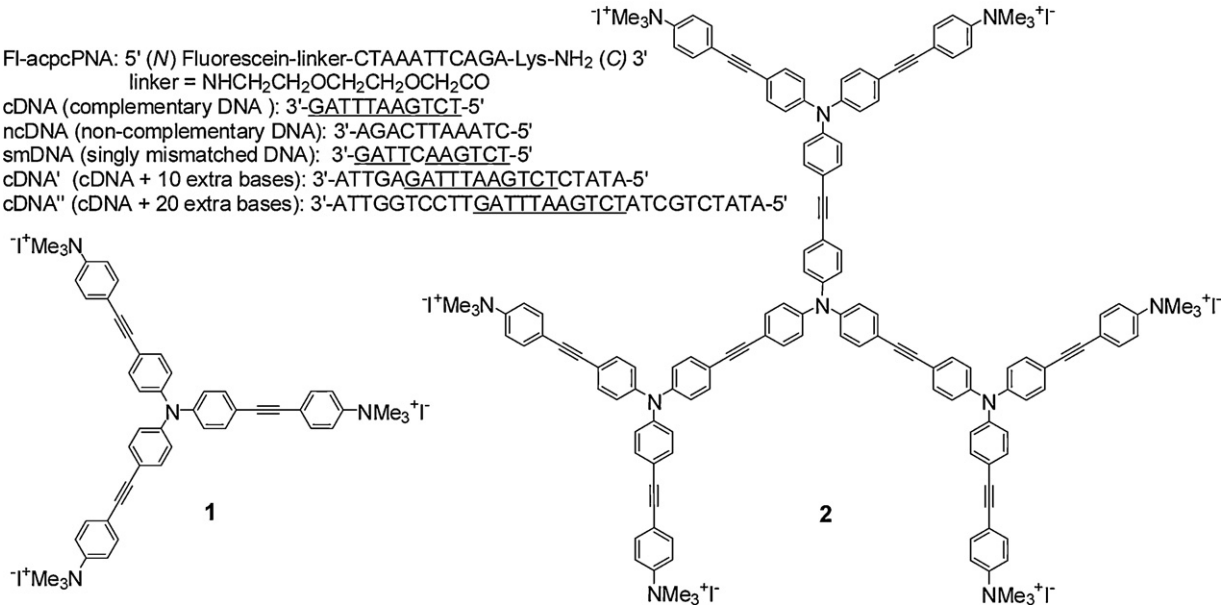


Fig. 1. Proposed detection of DNA/PNA hybridization via FRET from polycationic dendritic fluorophores $\mathbf{D^{n+}}$ (**1** and **2**) including their chemical structures and sequences of Fl-acpcPNA and DNAs used.

a class of polycationic fluorophores with definite molecular sizes and charges to be used as fluorescent transducers. Dendrimers are good candidates as their sizes and shapes can be controlled via convergent synthetic approach [27–30]. Recently, applications of dendritic phenylene-ethynylene polyelectrolytes as fluorescent signal transducer in sensing systems have been reported [31–36]. In this present work, we investigated the use of polycationic phenylene–ethynylene dendrimers **1** and **2** (Fig. 1) as a FRET donor in the detection of DNA sequence via the PNA/DNA hybridization principle. The specific PNA used in this work is a conformational constrained fluorescein-labeled pyrrolidinyl PNA (Fl-acpcPNA) derived from proline/2-aminocyclopentanecarboxylic acid backbone which exhibited superior specificity in DNA hybridization than the classical PNA [37,38].

2. Experimental

2.1. Chemicals and materials

Dendritic cationic fluorophores, **1** and **2**, were prepared as described in our previously reported procedures [34]. The acpcPNA probe was labeled at the *N*-terminus by 5(6)-carboxyfluorescein *N*-hydroxysuccinimidyl ester via an aminoethoxyethoxyacetyl spacer (–$NHCH_2CH_2OCH_2CH_2OCH_2CO$–) and was purified by HPLC after removal from the solid support [37,38]. HPLC-purified DNA oligo-nucleotides were purchased from Biodesign-Co., Ltd. (Thailand). Reagent grade formamide and *N*-methyl-2-pyrrolidinone were purchased from Merck and used without further purification.

2.2. Instruments

The UV–vis absorption spectra were recorded on a Varian Cary 50 UV–vis spectrophotometer. Fluorescence measurements were carried out with solution samples in a 3 mL quartz cuvette at room temperature using a Varian Cary Eclipse spectrofluorometer at 90° detection angle. The FRET ratios were calculated as the fluorescent intensity of the acceptor (fluorescein) over the intensity of the donor (**2**).

2.3. PNA/DNA sample preparation

Solutions of Fl-acpcPNA (0.6 μM) and DNA (0.6 μM) in 10 mM phosphate saline buffer pH 6.9 were mixed and allowed for hybridization at room temperature for overnight to provide a 0.3 μM Fl-acpcPNA/DNA duplex stock solution. For the fluorescence measurement, this stock solution was mixed with the fluorophore solution and the total volume was adjusted by 10 mM phosphate saline buffer pH 6.9, to give the final concentration of 1.0 μM for the fluorophore 0.1 μM for the DNA and PNA.

3. FRET experiments

To 1 mL of Fl-acpcPNA or Fl-acpcPNA/DNA stock solution (0.3 μM) was added 30 μL of **1** or **2** solution (100 μM), and the designated amount of 1-methyl-pyrrolidinone (NMP) or formamide at room temperature (25 °C). The final volume of the mixture was adjusted to 3 mL by 10 mM phosphate saline buffer pH 6.9 to afford the final concentration of 1 μM for the fluorophores and 0.1 μM for PNA and DNA. After the solution was thoroughly mixed for 1 min, the emission spectrum (410–700 nm) was recorded at room temperature with an excitation wavelength of 400 nm. The FRET signal and ratio were observed as the intensity of the fluorescein band peaked at 530 nm from the spectrum with the donor peak at 440 nm normalized.

4. Results and discussion

The basis of this work, as illustrated in Fig. 1, relies on the assumption that there is no significant interaction between the cationic dendritic fluorophores $\mathbf{D^{n+}}$ (**1** or **2**) and the electrostatically neutral Fl-acpcPNA. The addition of ssDNA with a complementary base sequence (cDNA) should give rise to a formation of a negatively charged DNA/PNA duplex, which in turn preferentially bind with the cationic dendrimer as a result of Coulombic interaction. A close proximity between $\mathbf{D^{n+}}$ and the fluorescein moiety on the acpcPNA should result in a FRET signal observed as enhanced fluorescein emission and reduced $\mathbf{D^{n+}}$ emission. On the other hands, if the DNA sequence has a non-complementary base sequence (ncDNA), there should be only the DNA/$\mathbf{D^{n+}}$ complexation, which gives no FRET signal.

The emission spectra of **1** and **2** along with the absorption and emission spectra of Fl-acpcPNA are shown in Fig. 2. Although the emission band of **1** has an ideal full overlapping integral with the absorption spectrum of Fl-acpcPNA, its broad emission band also cover a large portion of Fl-acpcPNA emission band. This precludes an efficient function of **1** as a FRET donor because the FRET signal detected from fluorescein emission would be greatly interfered by the donor emission. On the other hand, the emission band of the first generation dendrimer **2** advantageously appears at a significantly shorter wavelength probably due to its slower geometrical relaxation as a result of the bulkier dendritic arms. With a shorter emission wavelength, the overlapping area between the emission band of **2** and Fl-acpcPNA is considerably reduced and thus dendrimer **2** is a better choice as a FRET donor for Fl-acpcPNA. In addition, the higher number of positive charge (6+) on **2** should also provide a stronger electrostatic interaction with the PNA/DNA duplex that in turn enhance the efficiency of the FRET process.

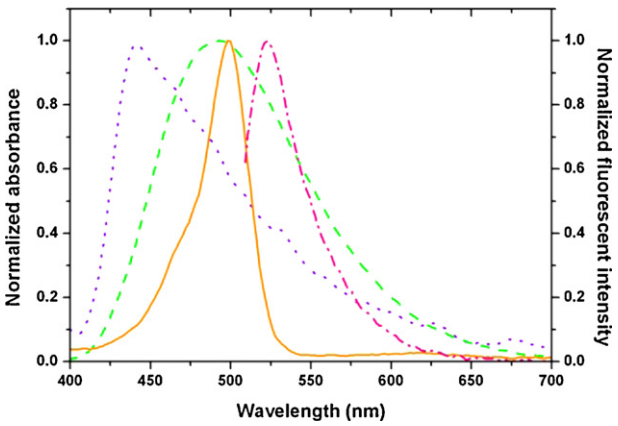


Fig. 2. Absorption spectrum of Fl-acpcPNA (–) and emission spectra of **1** (- - -), **2** (—) and Fl-acpcPNA (-·-).

Our initial FRET measurements showed that the use of **1** as the energy donor failed to give any distinguishable FRET signal even in the presence of the complementary DNA (Fig. 3a). On the other hand, the experiment with dendrimer **2** showed promising results. The strong emission signal of fluorescein was observed at 530 nm, especially for the system with cDNA (Fig. 3b). For the system with ncDNA, a much weaker FRET signal was observed. Interestingly, we observed a relatively strong FRET signal from the solution of only **2** and Fl-acpcPNA in the absence of any DNA. This result is somewhat deviate from our postulation proposed in Fig. 1 and it indicates a significant interaction between the donor and Fl-acpcPNA itself. This undesirable non-specific FRET signal may result from a relatively strong hydrophobic interaction between the dendritic core of **2** and the PNA backbone. Drastic reduction of the FRET signal in the presence of ncDNA implies that the Coulombic interaction of ncDNA with **2** is likely to be much stronger than the hydrophobic interaction between **2** and Fl-acpcPNA.

Recently, formamide has been successfully used as an additive to reduce non-specific hydrophobic interaction between DNA and PNA [39] while *N*-methylpyrrolidinone (NMP) has been used to reduce such interaction between DNA and cationic polyelectrolyte fluorophore [40]. These additives are known for their ability to

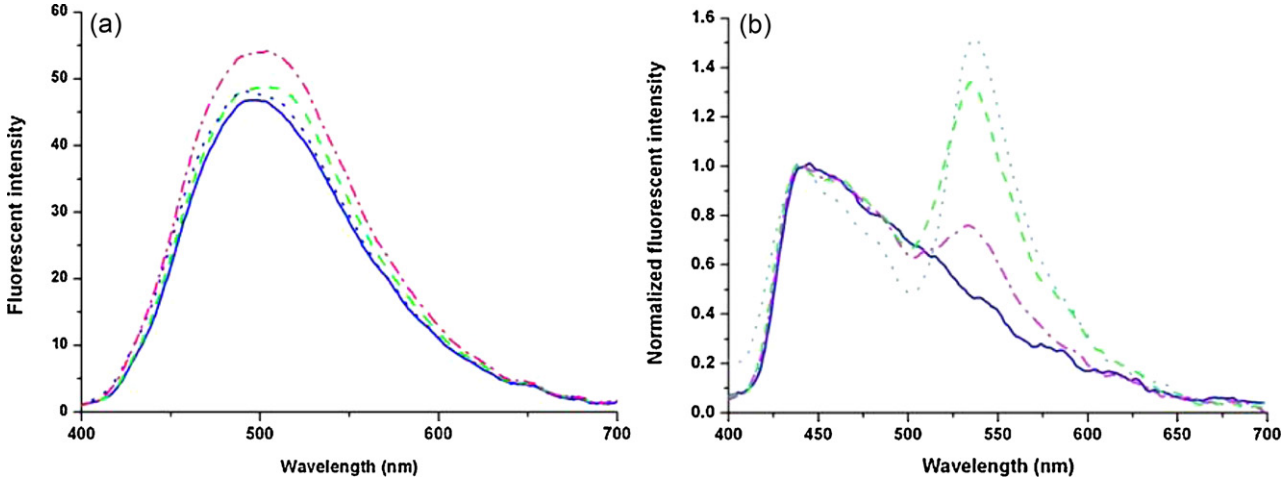


Fig. 3. (a) Emission spectra of **1** (–), **1**:Fl-acpcPNA (- - -), **1**:Fl-acpcPNA:cDNA (···) and **1**:Fl-acpcPNA:ncDNA (-·-) in phosphate saline buffer pH 6.9; [**1**] = 1.0 μM; [Fl-acpcPNA] = [DNA] = 0.1 μM. (b) Emission spectra of **2** (–), **2**:Fl-acpcPNA (- - -), **2**:Fl-acpcPNA:cDNA (···) and **2**:Fl-acpcPNA:ncDNA (-·-) in phosphate saline buffer pH 6.9; [**2**] = 1.0 μM; [Fl-acpcPNA] = [DNA] = 0.1 μM.

Table 1
Effects of additives on FRET ratios.

Additive	**2** + Fl-acpcPNA	**2** + Fl-acpcPNA + cDNA	**2** + Fl-acpcPNA + ncDNA
None	1.23 ± 0.14	1.40 ± 0.13	0.72 ± 0.11
Formamide (20%, v/v)	1.34 ± 0.16	1.66 ± 0.15	1.03 ± 0.09
NMP (20%, v/v)	0.58 ± 0.05	0.90 ± 0.07	0.47 ± 0.01
NMP (10%, v/v)	0.39 ± 0.15	1.42 ± 0.13	0.26 ± 0.18

solvate the PNA and DNA chain primarily via hydrogen bonding, which consequently reduce the hydrophobic interaction. In our tests, Table 1 shows that formamide cannot reduce the FRET ratio observed in the system containing only **2** and Fl-acpcPNA while NMP drastically decreases the FRET ratio of this system. At 10% (v/v) of NMP, the non-specific FRET ratio decreases without deteriorating the FRET signal generated from the desired Fl-acpcPNA/cDNA hybridization. Importantly, the FRET ratio of the PNA/ncDNA pair is also significantly reduced by NMP. Accordingly, the cDNA gives remarkably higher FRET signal than those of the ncDNA and the non-specific background in the presence of 10% (v/v) NMP (Fig. 4).

The effect of donor/acceptor ratio in the FRET process was studied at constant concentration of Fl-acpcPNA at 0.1 μM in the presence of 10% (v/v) NMP. The concentration of **2** was varied as 0.5, 1.0, and 1.5 μM. We compared the FRET ratios obtained from each condition in the presence of cDNA, ncDNA, and single mismatched DNA (smDNA), as well as in the absence of DNA. The plot in Fig. 5 indicates that the 1:10 M ratio of Fl-acpcPNA/**2** provide the best detection of cDNA while the non-specific signal, observed in the absence of DNA, is suppressed at a much lower level. This optimum ratio is probably governed by the countered balance between two concentration effects *i.e.* the increases of donor/acceptor complexation and the fluorescence background of **2**. It is also important to note that the cDNA (fully complementary DNA) can be clearly distinguished from the smDNA (singly mismatched DNA) at this optimal ratio.

We next investigated the effect of target DNA chain length on FRET efficiency by using 21-mer (cDNA′) and 31-mer DNA (cDNA″). Both cDNA′ and cDNA″ contain the complementary base sequence in the middle with extra base units extended from its both ends. The results are encouraging that the FRET ratio with cDNA′ and cDNA″ are successively higher than that in the case with cDNA (Fig. 6) and that the target DNAs with extra hanging nucleotide sequences is more likely the case encountered in real applications involving PCR-amplified or genomic DNA targets. The enhanced FRET signals by longer DNA chains highlight the importance of electrostatic interaction between the segment of DNA/PNA duplex and the cationic dendrimer in the FRET process.

The FRET ratio of the sensing system increased with the cDNA and a plot between corrected FRET ratio ($F_{(530)}$–$F_{0(530)}$) against concentration of cDNA yielded a linear line as shown in Fig. 7. The linear response suggests that the sensing system should be applicable for quantification of a designated DNA sequence. Fig. 7 also showed that the detection limit of this system is below 1 nM.

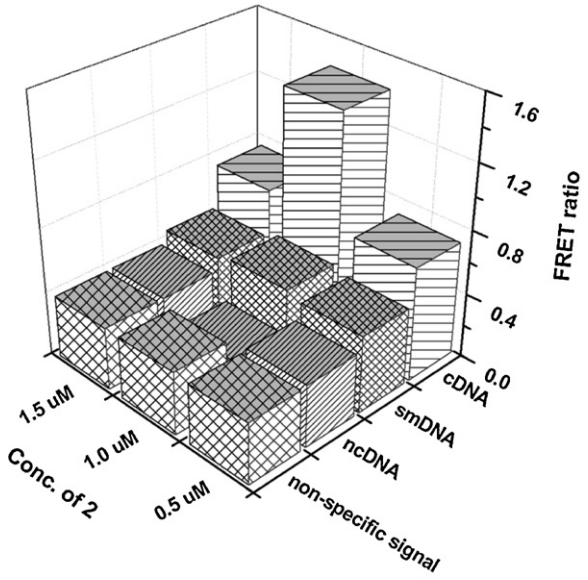


Fig. 5. FRET ratios obtained upon the addition of DNA (0.1 μM) to solutions containing varied concentrations of **2** and fixed Fl-acpcPNA concentration (0.1 μM).

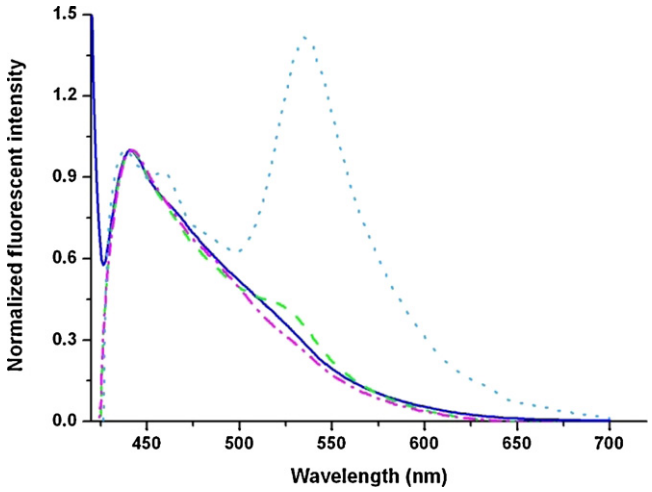


Fig. 4. Emission spectra of **2** (–), **2**:Fl-acpcPNA (- - -), **2**:Fl-acpcPNA:cDNA (···) and **2**:Fl-acpcPNA:ncDNA (-·-) in panel (a) phosphate saline buffer pH 6.9 with 10% (v/v) NMP [**2**] = 1.0 μM; [Fl-acpcPNA] = [DNA] = 0.1 μM.

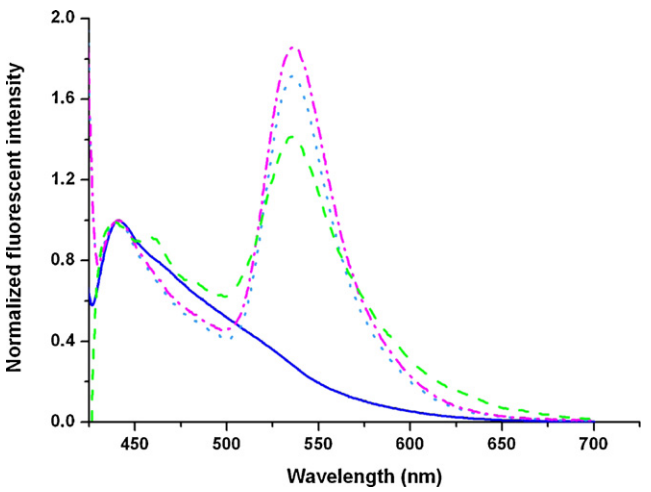


Fig. 6. Emission spectra of **2** (–), **2**:Fl-acpcPNA:cDNA (- - -), **2**:Fl-acpcPNA:cDNA′ (···) and **2**:Fl-acpcPNA:cDNA″ (-·-) in phosphate saline buffer pH 6.9 in the presence of 10% (v/v) NMP. [**2**] = 1.0 μM; [Fl-acpcPNA] = 0.1 μM; [DNA] = 0.1 μM.

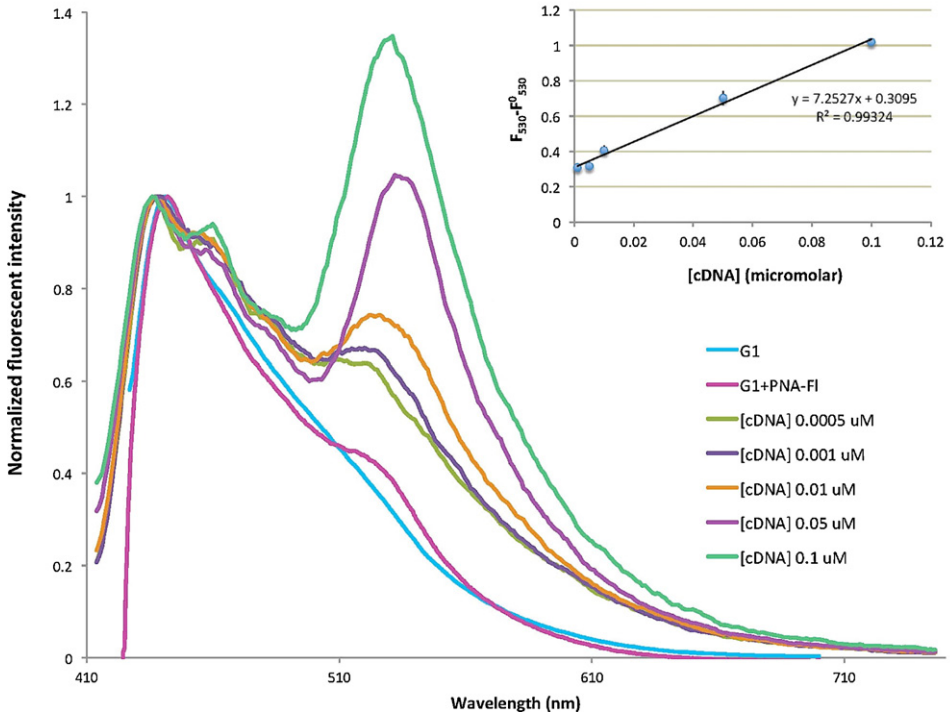


Fig. 7. Emission spectra and Linear plot between corrected FRET ratio ($F_{(530)}$–$F_{0(530)}$) and concentration of cDNA in phosphate saline buffer pH 6.9 with 10% (v/v) NMP [2] = 1.0 μM; [Fl-acpcPNA] = 0.1 μM.

5. Conclusion

In conclusion, we have demonstrated a detection of DNA hybridization by Förster resonance energy transfer (FRET) employing a cationic phenyleneethynylene dendritic fluorophore as a FRET donor and a fluorescein-labeled pyrrolidinyl peptide nucleic acid (Fl-acpcPNA) as the energy acceptor. The first generation fluorescent dendrimer with six positively charged ammonium peripheries provide adequate electrostatic interaction and spectral overlap resulted in readily distinguishable signals for the cases with complementary and non-complementary DNA. The background signal, which may be caused by the hydrophobic interaction between the inner part of the fluorophore and the peptide backbone of the acpcPNA could be eliminated by the addition of *N*-methyl-2-pyrrolidinone (NMP). Under optimum sensing condition, the sensing system readily detected fully complementary sequence DNA at submicromolar concentration level and distinguished it from the DNA with a single mismatch base. The cationic phenyleneethynylene dendrimer showed even higher FRET signal for detecting the target DNAs with extra hanging nucleotide sequences that should be useful for detecting PCR-amplified or genomic DNA targets in real applications.

Acknowledgement

This work is part of the Project for Establishment of Comprehensive Center for Innovative Food, Health Products and Agriculture supported by the Thai government stimulus package 2 (TKK2555, SP2) and receives financial support from the Thailand Research Fund (RTA5280002), the National Nanotechnology Center, NSTDA (NN-B-22-FN9-10-52-06), the 90th Anniversary of Chulalongkorn University Fund, the National Research University of CHE and the Ratchadaphiseksomphot Endowment Fund (AM1006A). KV thanks the Faculty of Science, Chulalongkorn University (A1B1-5) for her research fellowship. We also would like to thank Ms. Chalothorn Boonlua for Fl-acpcPNA samples.

References

[1] J. Zhu, Y. Lu, C. Deng, G. Huang, S. Chen, S. Xu, Y. Lv, K. Mitchelson, J. Cheng, Anal. Chem. 82 (2010) 5304–5312.
[2] I. Saaem, K-S. Ma, A.N. Marchi, T.H. LaBean, J. Tian, ACS Appl. Mater. Interfaces 2 (2010) 491–497.
[3] K. Boeneman, J.R. Deschamps, S. Buckhout-White, D.E. Prasuhn, J.B. Blanco-Canosa, P.E. Dawson, M.H. Stewart, K. Susumu, E.R. Goldman, M. Ancona, I.L. Medintz, ACS Nano 4 (2010) 7253–7266.
[4] A. Baeissa, N. Dave, B.D. Smith, J. Liu, ACS Appl. Mater. Interfaces 2 (2010) 3594–3600.
[5] R. Kanjanawarut, X. Su, Anal. Chem. 81 (2009) 6122–6129.
[6] E.G. Hvastkovs, D.A. Buttry, Analyst 135 (2010) 1817–1829.
[7] J.-Y. Park, S.-M. Park, Sensors 9 (2009) 9513–9532.
[8] X. Zhang, K. Jiao, S. Liu, Y. Hu, Anal. Chem. 81 (2009) 6006–6012.
[9] S. Ekgasit, G. Stengel, W. Knoll, Anal. Chem. 76 (2004) 4747–4755.
[10] B.P. Nelson, T.E. Grimsrud, M.R. Liles, R.M. Goodman, R.M. Corn, Anal. Chem. 73 (2001) 1–7.
[11] J.B. LePecq, C. Paoletti, J. Mol. Biol. 27 (1967) 87–106.
[12] J.T. Petty, J.A. Bordelon, M.E. Robertson, J. Phys. Chem. B 104 (2000) 7221–7227.
[13] R. Flehr, A. Kienzler, W. Bannwarth, M.U. Kumke, Bioconjugate Chem. 21 (2010) 2347–2354.
[14] Y.-N. Teo, E.T. Kool, Bioconjugate Chem. 20 (2009) 2371–2380.
[15] G. Liang, A.S. Susha, A.A. Lutich, F.D. Stefani, J. Feldmann, A.L. Rogach, ACS Nano 3 (2009) 4127–4131.
[16] R.R. Nayak, O.K. Nag, M. Kang, Y. Jin, H. Suh, K. Lee, H.Y. Woo, Macromol. Rapid Commun. 30 (2009) 633–638.
[17] B.S. Gaylord, A.J. Heeger, G.C. Bazan, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 10954–10957.
[18] B.S. Gaylord, A.J. Heeger, G.C. Bazan, J. Am. Chem. Soc. 125 (2003) 896–900.
[19] B.S. Gaylord, M.R. Massie, S.C. Feinstein, G.C. Bazan, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 34–39.
[20] E. Uhlman, A. Peyman, G. Breipohl, D.W. Will, Angew. Chem. Int. Ed. 37 (1998) 2796–2823.
[21] P.E. Nielsen, Acc. Chem. Res. 32 (1999) 624–630.
[22] B. Falkiewicz, Acta Biochim. Pol. 46 (1999) 509–529.
[23] K.N. Ganesh, P.E. Nielsen, Curr. Org. Chem. 4 (2000) 931–943.
[24] D.T. McQuade, A.E. Pullen, T.M. Swager, Chem. Rev. 100 (2000) 2537–2574.
[25] S.W. Thomas, G.D. Joly, T.M. Swager, Chem. Rev. 107 (2007) 1339–1386.
[26] C. Chi, A. Mikhailovsky, G.C. Bazan, J. Am. Chem. Soc. 129 (2007) 11134–11145.
[27] C.J. Hawker, J.M.J. Fréchet, J. Am. Chem. Soc. 112 (1990) 7638–7647.
[28] F. Zeng, S.C. Zimmerman, Chem. Rev. 97 (1997) 1681–1712.
[29] S.M. Grayson, J.M.J. Fréchet, Chem. Rev. 101 (2001) 3819–3867.
[30] P. Furuta, J.M.J. Fréchet, J. Am. Chem. Soc. 125 (2003) 13173–13181.

[31] B. Sandanaraj, R. Demont, S. Aathimanikandan, E.N. Savariar, S. Thayumanavan, J. Am. Chem. Soc. 128 (2006) 10686–10687.
[32] S. Jiwpanich, B.S. Sandanaraj, S. Thayumanavan, Chem. Commun. 80 (2009) 6–808.
[33] N. Niamnont, W. Siripornnoppakhun, P. Rashatasakhon, M. Sukwattanasinitt, Org. Lett. 11 (2009) 2768–2771.
[34] N. Niamnont, R. Mungkarndee, I. Techakriengkrai, P. Rashatasakhon, M. Sukwattanasinitt, Biosens. Bioelectron. 26 (2010) 863–867.
[35] B.W. Davis, N. Niamnont, C.D. Hare, M. Sukwattanasinitt, Q. Cheng, ACS Appl. Mater. Interfaces 2 (2010) 1798–1803.
[36] B.W. Davis, N. Niamnont, R. Dillon, B. Christopher, M. Sukwattanasinitt, Q. Cheng, Langmuir 27 (2011) 6401–6408.
[37] T. Vilaivan, C. Srisuwannaket, Org. Lett. 8 (2006) 1897–1900.
[38] C. Ananthanawat, T. Vilaivan, V.P. Hoven, X. Su, Biosens. Bioelectron. 25 (2010) 1064–1069.
[39] B. Boontha, J. Nakkuntod, N. Hirankarn, P. Chaumpluk, T. Vilaivan, Anal. Chem. 80 (2008) 8178–8186.
[40] F. Xia, X. Zou, R. Yang, Y. Xiao, D. Kang, A. Valle-Belisle, X. Gong, A.J. Heeger, K.W. Plaxco, J. Am. Chem. Soc. 132 (2010) 1252–1254.