

# Cationic Conjugated Polyelectrolyte/Molecular Beacon Complex for Sensitive, Sequence-Specific, Real-Time DNA Detection

Xuli Feng, Xinrui Duan, Libin Liu, Lingling An, Fude Feng, and Shu Wang\*

Beijing National Laboratory for Molecular Sciences, Key Laboratory of Organic Solids, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, PR China

Received September 6, 2008. Revised Manuscript Received September 17, 2008

A new fluorescence method has been developed for DNA detection at room temperature in a sensitive, selective, economical, and real-time manner that interfaces the superiority of a molecular beacon in mismatch discrimination with the light-harvesting property of water-soluble conjugated polyelectrolytes. The probe solution contains a cationic conjugated polyelectrolyte (PFP-NMe<sub>3</sub><sup>+</sup>), a molecular beacon with a five base pairs double-stranded stem labeled at the 5'-terminus with fluorescein (DNA<sub>P</sub>-Fl), and ethidium bromide (EB, a specific intercalator of dsDNA). The electrostatic interactions between DNA<sub>P</sub>-Fl and PFP-NMe<sub>3</sub><sup>+</sup> keep them in close proximity, facilitating the fluorescence resonance energy transfer (FRET) from PFP-NMe<sub>3</sub><sup>+</sup> to fluorescein. Upon adding a complementary strand to the probe solution, the conformation of DNA<sub>P</sub>-Fl transits into dsDNA followed by the intercalation of EB into the grooves. Two-step FRET, from PFP-NMe<sub>3</sub><sup>+</sup> to DNA<sub>P</sub>-Fl (FRET-1), followed by FRET from DNA<sub>P</sub>-Fl to EB (FRET-2) takes place. In view of the observed fluorescein or EB emission changes, DNA can be detected in aqueous solution. Because the base mismatch in target DNA inhibits the transition of DNA<sub>P</sub>-Fl from the stem-loop to duplex structure, single nucleotide mismatch can be clearly detected.

## Introduction

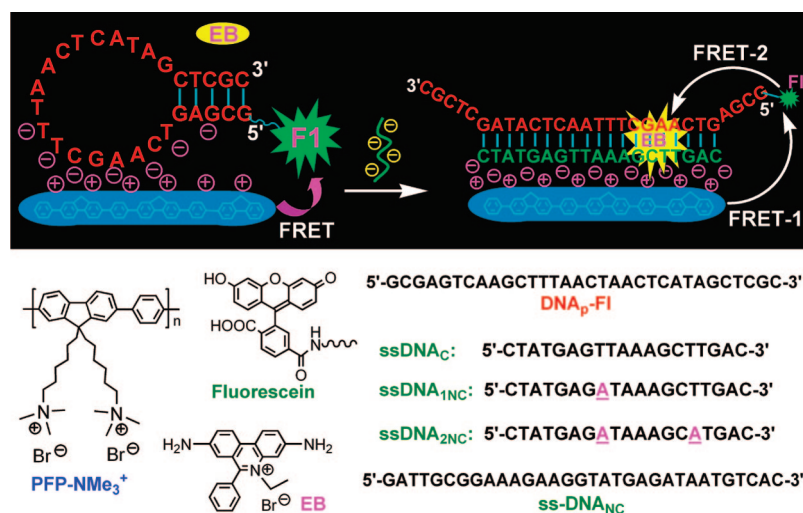
The sequence-specific detection of DNA plays a significant role in many areas, such as clinical diagnosis, gene expression analysis, and biomedical studies.<sup>1–4</sup> Consequently, various optical and electrochemical methods have been reported.<sup>5–8</sup> Among them, molecular beacons, which are composed of a hairpin like DNA stem-loop structure, have been reported to have excellent specificity in the detection of nucleic acid sequences.<sup>9,10</sup> Because of their superiority in mismatch discrimination, molecular beacons have been widely used to detect single-nucleotide mismatch and conformational changes in quantitative PCR<sup>11</sup> and microarray-based biosensors.<sup>12–15</sup> Although these methods can achieve excellent selectivity, their effectiveness is often limited by the sensitivity of the fluorescent reporter.<sup>16</sup> Additionally, the molecular beacon technique requires doubly labeled DNA probes, which increases the complexity and cost of probe design. There is thus a requirement for a sensitive, cost-effective molecular beacon method for DNA detection.

In recent years, conjugated polyelectrolytes (CPs) that provide amplified signals of hybridization events have been widely employed to detect DNA.<sup>17–25</sup> In comparison to small-molecule-based assays, the conjugated polyelectrolytes collect the action of a large number of absorbing units, and the transfer of excitation energy along the whole backbone to the chromophore reporter results in the amplification of fluorescence signals, which improves the detection sensitivity significantly.<sup>26–28</sup> In these assays, the CPs transduce the hybridization event of single-stranded probe and a target DNA to the optical signal with high sensitivity. However, less of them show good selectivity at room temperature;<sup>22,29</sup> that is, a single-nucleotide mismatch in the target sequence is hardly distinguished as a result of the nonspecific electrostatic interactions between CPs and the probe DNA. To improve the selectivity, microspheres, higher temperature, and PNA probe-assisted DNA detection by using CPs have been developed;<sup>16,30–32</sup> however, they need multiple steps, sophis-

\* Corresponding author. E-mail: wangshu@iccas.ac.cn.

- (1) Taton, T. A.; Mirkin, C. A.; Letsinger, R. L. *Science* **2000**, *289*, 1757.
- (2) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078.
- (3) Storhoff, J. J.; Elghanian, R.; Mucic, R. C.; Ichevsky, O.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 1959.
- (4) Schork, N. J.; Fallin, D.; Lanchbury, J. S. *Clin. Genet.* **2000**, *58*, 250.
- (5) Ranasinghe, R. T.; Brown, T. *Chem. Commun.* **2005**, 5487.
- (6) Tyagi, S.; Kramer, F. R. *Nat. Biotechnol.* **1996**, *14*, 303.
- (7) Park, S. J.; Taton, T. A.; Mirkin, C. A. *Science* **2002**, *295*, 1503.
- (8) Fan, C.; Plaxco, K. W.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9134.
- (9) Dubertret, B.; Calame, M.; Libchaber, A. *J. Nat. Biotechnol.* **2002**, *19*, 365.
- (10) Bonnet, G.; Tyagi, S.; Libchaber, A.; Kramer, F. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6171.
- (11) Balashov, S. V.; Gardiner, R.; Park, S.; Perlin, D. S. *J. Clin. Microbiol.* **2005**, *43*, 214.
- (12) Steemers, F. J.; Ferguson, J. A.; Walt, D. R. *Nat. Biotechnol.* **2000**, *18*, 91.
- (13) Liu, X.; Tan, W. *Anal. Chem.* **1999**, *71*, 5054.
- (14) Liu, X. J.; Farmerie, W.; Schuster, S.; Tan, W. *Anal. Biochem.* **2000**, *283*, 56.
- (15) Liu, G.; Wan, Y.; Gao, V.; Zhang, J.; Wang, L.; Song, S.; Fan, C. *J. Am. Chem. Soc.* **2008**, *130*, 6820.
- (16) Gaylord, B. S.; Massie, M. R.; Feinstein, S. C.; Bazan, G. C. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 34.

- (17) Liu, B.; Bazan, G. C. *Chem. Mater.* **2004**, *16*, 4467.
- (18) Ho, H. A.; Najari, A.; Leclerc, M. *Acc. Chem. Res.* **2008**, *41*, 168.
- (19) Yang, C. Y. J.; Pinto, M. R.; Schanze, K. S.; Tan, W. H. *Angew. Chem., Int. Ed.* **2005**, *44*, 2572.
- (20) Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10954.
- (21) Lee, K.; Rouillard, J.-M.; Pham, T.; Gulari, E.; Kim, J. *Angew. Chem., Int. Ed.* **2007**, *46*, 4667.
- (22) Nilsson, K. P. R.; Inganäs, O. *Nat. Mater.* **2003**, *2*, 419.
- (23) He, F.; Tang, Y.; Wang, S.; Li, Y.; Zhu, D. *J. Am. Chem. Soc.* **2005**, *127*, 12343.
- (24) Duan, X.; Li, Z.; He, F.; Wang, S. *J. Am. Chem. Soc.* **2007**, *129*, 4154.
- (25) Ho, H. A.; Dore, K.; Boissinot, M.; Bergeron, M. G.; Tanguay, R. M.; Boudreau, D.; Leclerc, M. *J. Am. Chem. Soc.* **2005**, *127*, 12673.
- (26) McQuade, D. T.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000**, *100*, 2537.
- (27) Swager, T. M. *Acc. Chem. Res.* **1998**, *31*, 201.
- (28) Thomas, S. W., III; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007**, *107*, 1339.
- (29) Lee, K.; Maisel, K.; Rouillard, J.-M.; Gulari, E.; Kim, J. *Chem. Mater.* **2008**, *20*, 2848.
- (30) Kushon, S. A.; Ley, K. D.; Bradford, K.; Jones, R. M.; McBranch, D.; Whitten, D. *Langmuir* **2002**, *18*, 7245.
- (31) Xu, H.; Wu, H.; Huang, F.; Song, S.; Li, W.; Cao, Y.; Fan, C. *Nucleic Acids Res.* **2005**, *33*, e83.
- (32) Ho, H. A.; Boissinot, M.; Bergeron, M. G.; Corbeil, G.; Doré, K.; Boudreau, D.; Leclerc, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 1548.

Scheme 1. Schematic Representation of the DNA Detection and Chemical Structures of PFP-NMe<sub>3</sub><sup>+</sup>, Fluorescein, EB, and the DNAs<sup>a</sup>

<sup>a</sup> DNA<sub>P-FI</sub> is the probe; ssDNA<sub>C</sub> is complementary to DNA<sub>P-FI</sub>; ssDNA<sub>1NC</sub> and ssDNA<sub>2NC</sub> are strands that are complementary to DNA<sub>P-FI</sub> with one- and two-base mismatches (highlighted in purple); ssDNA<sub>NC</sub> is fully noncomplementary to DNA<sub>P-FI</sub>.

ticated process, or expensive PNA. Our previous works show that the single-nucleotide mismatch detection of DNA can be realized by a DNA conformation transition<sup>33</sup> or a fluorescence melting curve technique.<sup>34</sup> However, these methods are used to probe the G-rich structure, which lacks universality, or are needed to raise the temperature, which makes the assays cumbersome. To overcome these disadvantages, recently we used target-induced DNA strand displacement to improve the selectivity of the CP-based DNA sensors;<sup>35</sup> however, additional endonuclease or DNazyme is involved in the detection process, which makes the assays complex and time-consuming. Recently, a molecular beacon—poly(phenylene ethynylene) conjugate has been prepared to detect DNA.<sup>19</sup> Herein, we present a new sensitive, selective, economical, fast fluorescent DNA assay that takes advantage of the superiority of molecular beacons in mismatch discrimination and the light-harvesting property of CPs. Our new assay method guarantees the fast detection of DNA at room temperature with excellent selectivity because of the conformational constraint of the stem-loop probe. Furthermore, the use of water-soluble conjugated polymers imparts high sensitivity to the sensor.

### Experimental Section

**Materials and Measurements.** PFP-NMe<sub>3</sub><sup>+</sup> was synthesized according to the procedure in the literature.<sup>36</sup> DNA<sub>P-FI</sub> and other oligonucleotides were purchased from Beijing Sunbiotech Co. Ltd. Their concentrations were determined by measuring the absorbance at 260 nm in a 250  $\mu$ L quartz cuvette. DNA<sub>P-FI</sub> was annealed at 80 °C for 20 min and slowly cooled to room temperature to get the hairpin structure. UV–vis absorption spectra were taken on a Hitachi U-3010 spectrophotometer. Fluorescence measurements were carried out in a 3 mL quartz cuvette at room temperature using a Hitachi F-4500 fluorometer equipped with a xenon lamp excitation source. All of the spectra were measured in phosphate buffer solution (50 mM, pH 7.8) at an excitation wavelength of 380 nm. Water was purified using a Millipore filtration system. A DYY-6C electrophoresis power supply and a DYCZ-24D polyacrylamide gel electrophoresis cell (Beijing Liuyi Instrument Factory) were used

in polyacrylamide gel electrophoresis (PAGE) analysis. The images were recorded by the ChemiDoc XRS system.

**DNA Detection.** To 1 mL of a phosphate buffer solution (50 mM, pH 7.8) was added DNA<sub>P-FI</sub>, PFP-NMe<sub>3</sub><sup>+</sup>, and EB ([PFP-NMe<sub>3</sub><sup>+</sup>] =  $1.0 \times 10^{-6}$  M in RUs, [DNA<sub>P-FI</sub>] =  $2.0 \times 10^{-8}$  M, and [EB] =  $3.0 \times 10^{-6}$  M) at room temperature, and then ssDNA<sub>C</sub> ([ssDNA<sub>C</sub>] =  $2.0 \times 10^{-8}$  M) was added. After the solution was mixed and incubated for 2 min, the fluorescence spectra were measured with an excitation wavelength of 380 nm at room temperature. In the case of DNA mismatch detection, the assays were performed under the same conditions as above except that target DNAs with one, two, and more base mismatches (named ssDNA<sub>1NC</sub>, ssDNA<sub>2NC</sub>, and ssDNA<sub>NC</sub>, respectively) were used instead of fully matched ssDNA<sub>C</sub>.

**Measurements of Kinetic Structural Transition of DNA<sub>P-FI</sub>.** To 1 mL of a phosphate buffer solution (50 mM, pH 7.8) was added DNA<sub>P-FI</sub>, PFP-NMe<sub>3</sub><sup>+</sup>, and EB ([PFP-NMe<sub>3</sub><sup>+</sup>] =  $1.0 \times 10^{-6}$  M in RUs, [DNA<sub>P-FI</sub>] =  $2.0 \times 10^{-8}$  M, and [EB] =  $3.0 \times 10^{-6}$  M) at room temperature. The emission maximum intensity of EB at 602 nm versus time was measured with an excitation wavelength of 380 nm at room temperature. After 50 s, ssDNA<sub>C</sub> ([ssDNA<sub>C</sub>] =  $2.0 \times 10^{-8}$  M) was injected, and the fluorescence measurement was continued over a time range of 300 s. The emission maximum intensity of F1 at 530 nm versus the hybridization time was also measured with the same procedures as used for EB. The curve of the FRET ratio ( $I_{602\text{ nm}}/I_{530\text{ nm}}$ ) as a function of time was then obtained.

For the mismatched DNAs, the assays were performed under the same conditions as above except for using target DNAs with one, two, and more base mismatches (named ssDNA<sub>1NC</sub>, ssDNA<sub>2NC</sub>, and ssDNA<sub>NC</sub>, respectively) instead of fully matched ssDNA<sub>C</sub>.

**Electrophoresis Analysis.** DNA<sub>P-FI</sub> samples before and after hybridization with ssDNA<sub>C</sub>, ssDNA<sub>1NC</sub>, ssDNA<sub>2NC</sub>, and ssDNA<sub>NC</sub> were respectively loaded onto 15% nondenaturing polyacrylamide gel in a 1 $\times$  TBE buffer (8.9 mM tris base, 8.9 mM boric acid, 0.2 mM EDTA, pH 7.9) followed by electrophoresis for 45 min at 100 V. After the gel was stained with SYBR Gold, a photograph was taken with the ChemiDoc XRS system.

### Results and Discussion

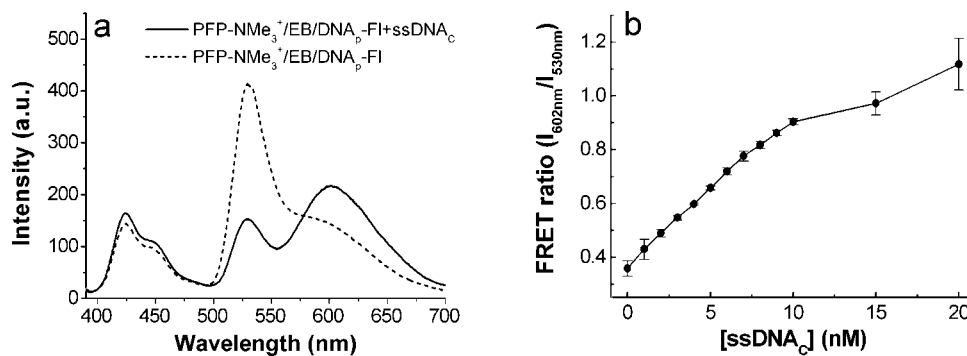
Our new assay strategy for DNA detection is illustrated in Scheme 1. Cationic poly[(9,9-bis(6'-N, N,N-trimethylammonium)hexyl)-fluorenylene phenylene dibromide] (PFP-NMe<sub>3</sub><sup>+</sup>)<sup>36</sup> is used as the conjugated polyelectrolyte in fluorescence resonance energy transfer (FRET) experiments. The molecular beacon DNA with a five base pairs double-stranded stem labeled with fluorescein at its 5'-terminus (DNA<sub>P-FI</sub>) acts as the probe. One

(33) He, F.; Tang, Y.; Yu, M.; Feng, F.; An, L.; Sun, H.; Wang, S.; Li, Y.; Zhu, D.; Bazan, G. C. *J. Am. Chem. Soc.* **2006**, *128*, 6764.

(34) Tian, N.; Tang, Y.; Xu, Q.; Wang, S. *Macromol. Rapid Commun.* **2007**, *28*, 729.

(35) He, F.; Feng, F.; Duan, X.; Wang, S.; Li, Y.; Zhu, D. *Anal. Chem.* **2008**, *80*, 2239.

(36) Stork, M.; Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. *Adv. Mater.* **2002**, *14*, 361.



**Figure 1.** (a) Fluorescence spectra of DNA<sub>p</sub>-FI in the presence of PFP-NMe<sub>3</sub><sup>+</sup> and EB before and after the addition of ssDNA<sub>C</sub> in phosphate buffer (50 mM, pH 7.8). [PFP-NMe<sub>3</sub><sup>+</sup>] = 1.0 × 10<sup>-6</sup> M RUs, [EB] = 3.0 × 10<sup>-6</sup> M, and [DNA<sub>p</sub>-FI] = [ssDNA<sub>C</sub>] = 2.0 × 10<sup>-8</sup> M. (b) FRET ratio as a function of ssDNA<sub>C</sub> concentration. [PFP-NMe<sub>3</sub><sup>+</sup>] = 1.0 × 10<sup>-6</sup> M RUs, [EB] = 3.0 × 10<sup>-6</sup> M, [DNA<sub>p</sub>-FI] = 2.0 × 10<sup>-8</sup> M, and [ssDNA<sub>C</sub>] = 0–2.0 × 10<sup>-8</sup> M. The excitation wavelength is 380 nm. Error bars represent the standard deviation of three experiments.

begins with a solution that contains the cationic PFP-NMe<sub>3</sub><sup>+</sup>, DNA<sub>p</sub>-FI, and ethidium bromide.<sup>37</sup> (EB is a specific intercalator of dsDNA.) Electrostatic interactions between DNA<sub>p</sub>-FI and PFP-NMe<sub>3</sub><sup>+</sup> keep them in close proximity, facilitating FRET from PFP-NMe<sub>3</sub><sup>+</sup> to fluorescein. Because the double-stranded stem of DNA<sub>p</sub>-FI is very short, EB can hardly intercalate into it. In this case, one can see only the emission of fluorescein. Upon adding a complementary strand to the assay solution containing PFP-NMe<sub>3</sub><sup>+</sup>, DNA<sub>p</sub>-FI, and EB, the conformation of DNA<sub>p</sub>-FI transits into dsDNA. Under these conditions, the EB can easily intercalate into the grooves of dsDNA. The excitation of PFP-NMe<sub>3</sub><sup>+</sup> at 380 nm leads to two-step FRET: from PFP-NMe<sub>3</sub><sup>+</sup> to DNA<sub>p</sub>-FI (FRET-1) and then from DNA<sub>p</sub>-FI to EB (FRET-2). Direct FRET from PFP-NMe<sub>3</sub><sup>+</sup> to EB is not favorable because of the nonoptimized transition dipole orientations between them within the PFP-NMe<sub>3</sub><sup>+</sup>/dsDNA(EB) complex.<sup>36</sup> Thus, the target DNA can be detected by fluorescence spectra in view of the observed fluorescein or EB emission changes in aqueous solutions. It is noted that the five base pairs double-stranded stem of the DNA<sub>p</sub>-FI probe is necessary for the target DNA assay. When a DNA<sub>p</sub>-FI probe containing a longer double-stranded stem (such as 12 base pairs) was used, the fluorescein or EB emission signal was hardly distinguished before and after hybridization of DNA<sub>p</sub>-FI with target DNA because the EB could intercalate into the double-stranded grooves in both cases (Figure S1).

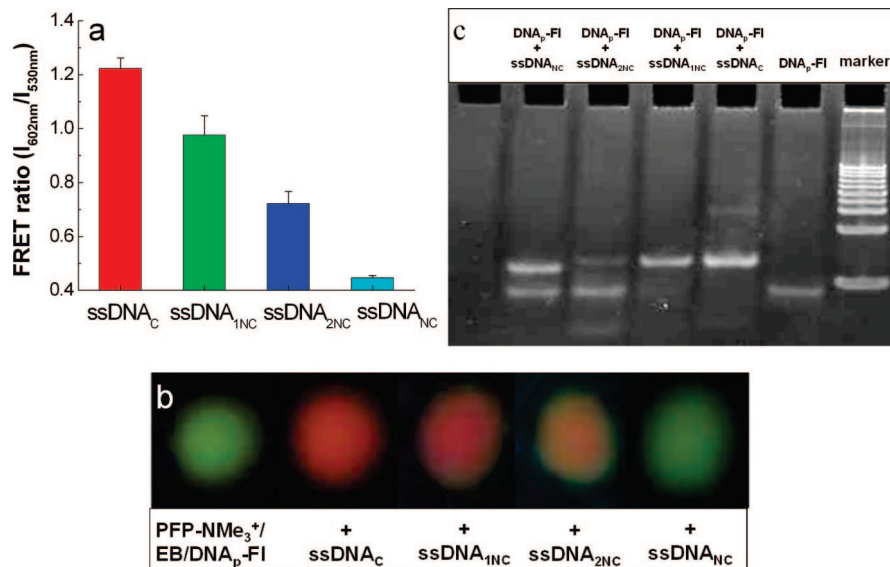
Figure 1a compares the emission spectra of PFP-NMe<sub>3</sub><sup>+</sup>/DNA<sub>p</sub>-FI/EB ([PFP-NMe<sub>3</sub><sup>+</sup>] = 1.0 × 10<sup>-6</sup> M in repeat units (RUs), [DNA<sub>p</sub>-FI] = 2.0 × 10<sup>-8</sup> M, and [EB] = 3.0 × 10<sup>-6</sup> M) in phosphate buffer solution (50 mM, pH 7.8) before and after the addition of complementary ssDNA<sub>C</sub> ([ssDNA<sub>C</sub>] = 2.0 × 10<sup>-8</sup> M). The excitation wavelength was chosen at the absorbance maximum of PFP-NMe<sub>3</sub><sup>+</sup> (380 nm), where no significant absorption by fluorescein and EB occurs. In the absence of ssDNA<sub>C</sub>, only FRET from PFP-NMe<sub>3</sub><sup>+</sup> to fluorescein is observed. Upon adding ssDNA<sub>C</sub> to the detection solution, the transition from the stem-loop to duplex structure takes place, followed by EB intercalation, and emission from EB is obviously observed. To investigate the dynamic range of the target DNA concentration in our experiments, the emission spectra of PFP-NMe<sub>3</sub><sup>+</sup>/DNA<sub>p</sub>-FI/EB with fixed concentrations in phosphate buffer ([PFP-NMe<sub>3</sub><sup>+</sup>] = 1.0 × 10<sup>-6</sup> M in RU, [DNA<sub>p</sub>-FI] = 2.0 × 10<sup>-8</sup> M, and [EB] = 3.0 × 10<sup>-6</sup> M) were measured by changing the concentration of added ssDNA<sub>C</sub> from 0 to 20 nM at an excitation wavelength of 380 nm. Figure 1b shows the FRET ratio of EB to fluorescein emission intensity ( $I_{602\text{ nm}}/I_{530\text{ nm}}$ ) as a function of

ssDNA<sub>C</sub> concentration. As the concentration of ssDNA<sub>C</sub> increases, the emission intensity of fluorescein at 530 nm decreases, and that of EB at 602 nm increases. It is noticeable that as low as 1 nM ssDNA<sub>C</sub> can obviously be detected using a standard Hitachi F-4500 fluorometer.

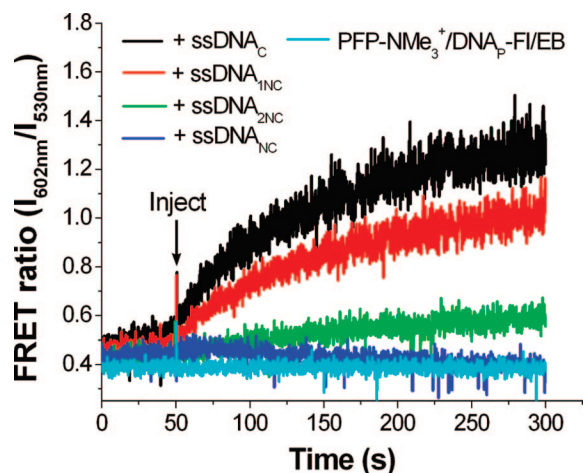
To evaluate the specificity of our assay method, the effect of base mismatch in the complementary strand on the FRET ratio ( $I_{602\text{ nm}}/I_{530\text{ nm}}$ ) was studied by adding ssDNA<sub>1NC</sub>, ssDNA<sub>2NC</sub>, and ssDNA<sub>NC</sub> to PFP-NMe<sub>3</sub><sup>+</sup>/DNA<sub>p</sub>-FI/EB solutions ([PFP-NMe<sub>3</sub><sup>+</sup>] = 1.0 × 10<sup>-6</sup> M in RUs, [DNA<sub>p</sub>-FI] = 2.0 × 10<sup>-8</sup> M, and [EB] = 3.0 × 10<sup>-6</sup> M) in phosphate buffer (50 mM, pH 7.8). As shown in Figure 2a, the FRET ratio ( $I_{602\text{ nm}}/I_{530\text{ nm}}$ ) follows the order ssDNA<sub>C</sub> > ssDNA<sub>1NC</sub> > ssDNA<sub>2NC</sub> > ssDNA<sub>NC</sub>. Increasing the number of mismatches inhibits the stem-loop to duplex structure transition of the DNA<sub>p</sub>-FI probe. This inhibition results from the conformational constraint of DNA<sub>p</sub>-FI, in which the presence of the stem makes it thermodynamically unfavorable for the binding of the mismatched sequence to the loop.<sup>10</sup> Figure 2b shows the images of PFP-NMe<sub>3</sub><sup>+</sup>/DNA<sub>p</sub>-FI/EB solutions before and after adding ssDNA<sub>C</sub>, ssDNA<sub>1NC</sub>, ssDNA<sub>2NC</sub>, and ssDNA<sub>NC</sub> that were dropped onto a glass slide under UV light with excitation at 365 nm. The base mismatch could be clearly distinguished with different emission colors. These results undoubtedly demonstrate that the new assay method has excellent selectivity and the single-nucleotide mismatch can be clearly detected in target DNA in aqueous solutions. The electrophoresis analysis was performed using a 15% nondenaturing polyacrylamide gel. As shown in Figure 2c, DNA<sub>p</sub>-FI itself moves as a single band, and the addition of fully complementary ssDNA<sub>C</sub> yields one slowly moving band, which indicates that the hybridization of DNA<sub>p</sub>-FI with ssDNA<sub>C</sub> forms a more stable DNA<sub>p</sub>-FI/ssDNA<sub>C</sub> duplex. It is noted that the intensity of the duplex band decreases as the number of mismatched bases in target DNA increases. These results also show that increasing the number of mismatches inhibits the stem-loop to duplex structure transition of the DNA<sub>p</sub>-FI probe, which clearly proves the results of fluorescence experiments.

The effect of base mismatch in the complementary strand on the kinetic structural transition of DNA<sub>p</sub>-FI was studied. In these experiments, the four phosphate buffer solutions (50 mM, pH 7.8) containing PFP-NMe<sub>3</sub><sup>+</sup>, DNA<sub>p</sub>-FI, and EB ([PFP-NMe<sub>3</sub><sup>+</sup>] = 1.0 × 10<sup>-6</sup> M in RUs, [DNA<sub>p</sub>-FI] = 2.0 × 10<sup>-8</sup> M, and [EB] = 3.0 × 10<sup>-6</sup> M) were prepared at room temperature. After being incubated for 50 s, ssDNA<sub>C</sub>, ssDNA<sub>1NC</sub>, ssDNA<sub>2NC</sub>, and ssDNA<sub>NC</sub> were injected into above solutions, respectively. The FRET ratio ( $I_{602\text{ nm}}/I_{530\text{ nm}}$ ) was then measured over a period of 0–300 s. As shown in Figure 3, the structural transition process

(37) Wang, S.; Gaylord, B. S.; Bazan, G. C. *J. Am. Chem. Soc.* **2004**, *126*, 5446.



**Figure 2.** (a) FRET ratio ( $I_{602\text{nm}}/I_{530\text{nm}}$ ) of PFP-NMe<sub>3</sub><sup>+</sup>/DNA<sub>P</sub>-FI/EB solutions in phosphate buffer (50 mM, pH 7.8) upon adding ssDNA<sub>C</sub>, ssDNA<sub>1NC</sub>, ssDNA<sub>2NC</sub>, and ssDNA<sub>NC</sub>. [PFP-NMe<sub>3</sub><sup>+</sup>] =  $1.0 \times 10^{-6}$  M in RUs, [DNA<sub>P</sub>-FI] = [ssDNA<sub>C</sub>] = [ssDNA<sub>1NC</sub>] = [ssDNA<sub>2NC</sub>] = [ssDNA<sub>NC</sub>] =  $2.0 \times 10^{-8}$  M, and [EB] =  $3.0 \times 10^{-6}$  M. The excitation wavelength is 380 nm. Error bars represent the standard deviation of three experiments. (b) Images of PFP-NMe<sub>3</sub><sup>+</sup>/DNA<sub>P</sub>-FI/EB solutions before and after adding ssDNA<sub>C</sub>, ssDNA<sub>1NC</sub>, ssDNA<sub>2NC</sub>, and ssDNA<sub>NC</sub> that were dropped onto a glass slide under UV light with excitation at 365 nm. (c) Electrophoresis analysis of DNA<sub>P</sub>-FI before and after adding ssDNA<sub>C</sub>, ssDNA<sub>1NC</sub>, ssDNA<sub>2NC</sub>, and ssDNA<sub>NC</sub>.



**Figure 3.** FRET ratio of  $I_{602\text{nm}}/I_{530\text{nm}}$  against the incubation time of DNA<sub>P</sub>-FI with its complementary ssDNA<sub>C</sub> and noncomplementary ssDNA<sub>1NC</sub>, ssDNA<sub>2NC</sub>, and ssDNA<sub>NC</sub>. [PFP-NMe<sub>3</sub><sup>+</sup>] =  $1.0 \times 10^{-6}$  M in RUs, [DNA<sub>P</sub>-FI] = [ssDNA<sub>C</sub>] = [ssDNA<sub>1NC</sub>] = [ssDNA<sub>2NC</sub>] = [ssDNA<sub>NC</sub>] =  $2.0 \times 10^{-8}$  M, and [EB] =  $3.0 \times 10^{-6}$  M. The excitation wavelength is 380 nm.

of DNA<sub>P</sub>-FI is very quick, and the FRET ratio ( $I_{602\text{nm}}/I_{530\text{nm}}$ ) rapidly reaches a plateau within  $\sim 4$  min. The transition rate follows the order ssDNA<sub>C</sub> > ssDNA<sub>1NC</sub> > ssDNA<sub>2NC</sub> > ssDNA<sub>NC</sub>. The kinetic measurements thus make it possible to probe the transition from stem-loop to duplex structure and detect target DNA with single-nucleotide mismatch discrimination in real time.

In conclusion, a new sensitive, selective, economical, fast fluorescence method has been developed for DNA detection that interfaces the superiority of a molecular beacon in mismatch discrimination with the light-harvesting property of water-soluble conjugated polyelectrolytes. A single-nucleotide mismatch can be clearly detected in target DNA. The new method does not require any denaturation steps and exhibits fast DNA detection at room temperature with excellent selectivity. The detection can be carried out under UV light, and no expensive detection instrumentation is needed. In comparison to the established molecular-beacon-based DNA detection systems, this technique requires only one fluorescent label on the probe. Additionally, the amplification of fluorescence signals from CCPs improves the detection sensitivity. Thus, the technique based on a conjugated polyelectrolyte and a molecular beacon is very promising for DNA detection with high sensitivity and selectivity.

**Acknowledgment.** We are grateful for financial support from the 100 Talents program of the Chinese Academy of Sciences, the National Natural Science Foundation of China (nos. 20725308, 20721061, and 20574073), the National High-Tech R&D Program (no. 2006AA02Z130), and the Major Research Plan of China (no. 2006CB932100).

**Supporting Information Available:** Fluorescence spectra of DNA<sub>P</sub>-FI containing a 16 base pairs double-stranded stem in the presence of PFP-NMe<sub>3</sub><sup>+</sup> and EB before and after the addition of complementary DNA in phosphate buffer. This material is available free of charge via the Internet at <http://pubs.acs.org>.

LA802932T