DNA Detection

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Ultrasensitive DNA Detection Using Photonic Crystals**

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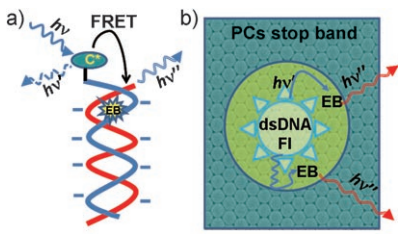
In recent years, there has been immense interest in the detection of DNA hybridization, which is very important for the diagnostics of genetic and pathogenic diseases.[1] High-sensitivity and high-selectivity detection is necessary for early disease diagnosis and treatment.[2] Most available methods involve molecular-fluorophore-based assays, in which the target DNA is hybridized with a specific base-sequence probe labeled with a radioisotope or a fluorophore.[3] In these DNA detection processes, the amplification steps are important to realize the ultimate in terms of sensitivity. These amplification systems include polymerase chain reaction (PCR) protocols and signal-amplification systems, such as fluorogenic substrate-active enzymes,[4] modified liposomes,[5] and nanoparticles.[6] However, the relatively complex instrument and specific reagents required restrict their practical application.

Fluorescence resonance energy transfer (FRET) between energy-donor and -acceptor pairs is a feasible approach to improve the sensitivity and selectivity of the assay.[7] In that way, the light-harvesting "antennas" can bring about the amplification of the biosensor signals. Heeger, Bazan, and co-workers have used light-harvesting cationic conjugated polyelectrolytes and shape-adaptable polymers for strand-specific DNA detection with high sensitivity.[8] Mattoussi and co-workers have enabled hybrid inorganic–bioreceptor sensing materials by using luminescent semiconductor quantum dots as chemical sensors, which allow the continuous monitoring of target (bio)chemical species in diverse environments.[9] However, these methods have difficulties in controlling the structure, size, and shape of the antennas and the energy-transfer efficiency is hampered as a result of the energy loss through the waveguide and diffusion. Therefore, it is desirable to develop facile methods that can efficiently enhance the optical signal based on FRET.

Photonic crystals (PCs) are promising materials for the amplification of spontaneous emission as well as the control of the propagation of light with minimal losses.[10] Colloidal PCs are economical three-dimensional PCs with feature sizes of the wavelength of light, which are made from self-assembled monodispersed colloidal spheres.[11] Many reports have revealed that spontaneous radiation from excited molecules can be strongly modified by embedding light sources in the PCs.[12] Vos and co-workers have demonstrated the enhanced and angle-dependent emission in PCs.[13] The groups of Furumi,[14] Foulger,[15] and Vardeny[16] have described dynamic tunable lasers by using a PC as an external resonator cavity.

In our previous work, we used PCs to enhance the emission density of organic dyes[17] and to achieve nonlinear emission.[18] In all of the reported demonstrations, the PCs are of great potential in modification of the spontaneous emission of active optical devices.[19] The superior properties of PCs create a new approach to amplifying biosensor signals and thereby to developing highly sensitive optical biosensors. Herein, we demonstrate an effective FRET-based DNA hybridization detection method by utilizing a PC to amplify the optical signal. The method has single-mismatch selectivity and a sensitivity of approximately 13.5 fM, which is hundreds of times greater than those obtained with conventional fluorophore-based methods.

Scheme 1 shows the DNA detection system with a PC. The PCs are made by self-assembly of monodispersed poly(styrene–methyl methacrylate–acrylic acid) [poly(St-MMA-AA)] spheres. The detection system consists of a fluorescein (Fl)-labeled DNA (DNA-Fl) probe and ethidium bromide (EB), which serve as the donor and acceptor, respectively. The DNA-Fl is obtained by introducing an Fl unit at the 5′ terminus of a single-stranded DNA (ssDNA) (ssDNA-Fl). Fl, with an absorption maximum at 488 nm and an emission maximum at 520 nm, is chosen because its emission overlaps with the absorption of EB. FRET from Fl to EB becomes thus energetically feasible.[20] EB is a well-known intercalator into double-stranded DNA (dsDNA). If the probe and target ssDNAs are complementary



Scheme 1. a) DNA sequence detection based on a FRET mechanism. b) Effect of the PC on FRET.

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(Scheme 1a), DNA duplex formation will ensue and EB will intercalate into the dsDNA, resulting in an increase in its fluorescence quantum yield.[21] Thus, EB emission upon FRET sensitization from the Fl can be anticipated. If the target ssDNA is not complementary to probe DNA, the formation of the dsDNA structure required for EB intercalation will not take place and FRET to EB is hardly detectable.

The sequences of the five ssDNAs studied—$ssDNA_P$-Fl corresponds to the probe strand, $ssDNA_C$ is a strand complementary to $ssDNA_P$-Fl, and $ssDNA_{1NC}$, $ssDNA_{3NC}$, and $ssDNA_{5NC}$ are three strands, respectively, contain one, three and five base-mismatches in comparison to $ssDNA_C$—are given in the Experimental Section. Our initial efforts concerned the amplification of the FRET efficiency by taking advantage of the effect of the PC so as to improve the sensitivity of the detection system. To evaluate the role of different stop bands on the FRET, different PC samples were fabricated and detected from monodispersed poly(St-MMA-AA) latex spheres. Herein, we report three typical PCs with diameters of 210 (PCI), 216 (PCII), and 270 nm (PCIII); their corresponding stop bands are 520, 550, and 622 nm, respectively.

Figure 1a shows the relevant absorption and emission spectra of the donor (dsDNA-Fl) and the acceptor (EB). The spectra were collected in a potassium phosphate–sodium hydroxide buffer solution (pH 7.4) at concentrations used in DNA hybridization protocols. It is known that the overlap and suitable orientation between the transition bands and the structures of the donor and acceptor are crucial to the FRET efficiency.[22] The molecular components of the assay were chosen to satisfy these optical requirements (see Figure S1 in the Supporting Information). The overlap integral provides the analytical expression for how the spectral overlap between the emission of the donor and the absorption of the acceptor influences the rate of transfer.

Figure 1b shows the emission of the detection system dsDNA-Fl/EB embedded in PCI at $[dsDNA\text{-}Fl] = 5.0 \times 10^{-8}$ M as a function of EB concentration. Comparison of the resulting fluorescence reveals a predominant effect on the emission dispersion properties of FRET between dsDNA-Fl and EB. The additions of EB cause a decrease in the emission intensities of dsDNA-Fl l with a concomitant increase in EB emission intensity. Once the concentration ratio of EB to dsDNA-Fl reaches approximately 28, the emission intensity of EB no longer increases.

In Figure 1c the emission spectra of dsDNA-Fl/EB ($\lambda_{ex} =$ 488 nm) within PCI and in solution are given. The EB emission intensity of dsDNA-Fl/EB immersed in PCI is about 14.3-fold greater than that of the same system directly excited in solution. The FRET ratio ($I_{EB}/I_{Fl} = 1.71$) in PCI is enhanced circa 2.5 times compared with that in solution ($I_{EB}/I_{Fl} = 0.69$). The results indicate that PCI can strongly improve the overall energy-transfer efficiency from Fl to EB. Here, I_{EB}/I_{Fl} gives the proportion of the maximum of the acceptor Fl emission and the donor EB emission to indicate the FRET efficiency. Within the usual FRET system in solution, FRET exhibits poor energy-transfer efficiency from the Fl to the EB emission without being modified. The low efficiency is the result of a certain degree of loss through diffusion and reabsorption in the energy-transfer process.

The poly(St-MMA-AA) PC with a diameter of 210 nm (see Figure S2 in the Supporting Information) is chosen as the optimized PC structure whose stop band ($\lambda = 520$ nm) just overlaps with the emission band of Fl as well as the absorption band of EB. It has been predicted that when a fluorescent molecule with a resonant transition within the frequency gap is placed in the photonic-band material, a photon bond state will be formed and the light within it will be strongly localized. The stop band of the as-prepared PCI will serve as the dielectric cavity and act as a local resonance mode for the emission propagation of the donor.

It is clearly seen in the spectra of the detection system within PCI (Figure 1c and d) that there is a moderate dip at the wavelength of 525 nm. The prohibited

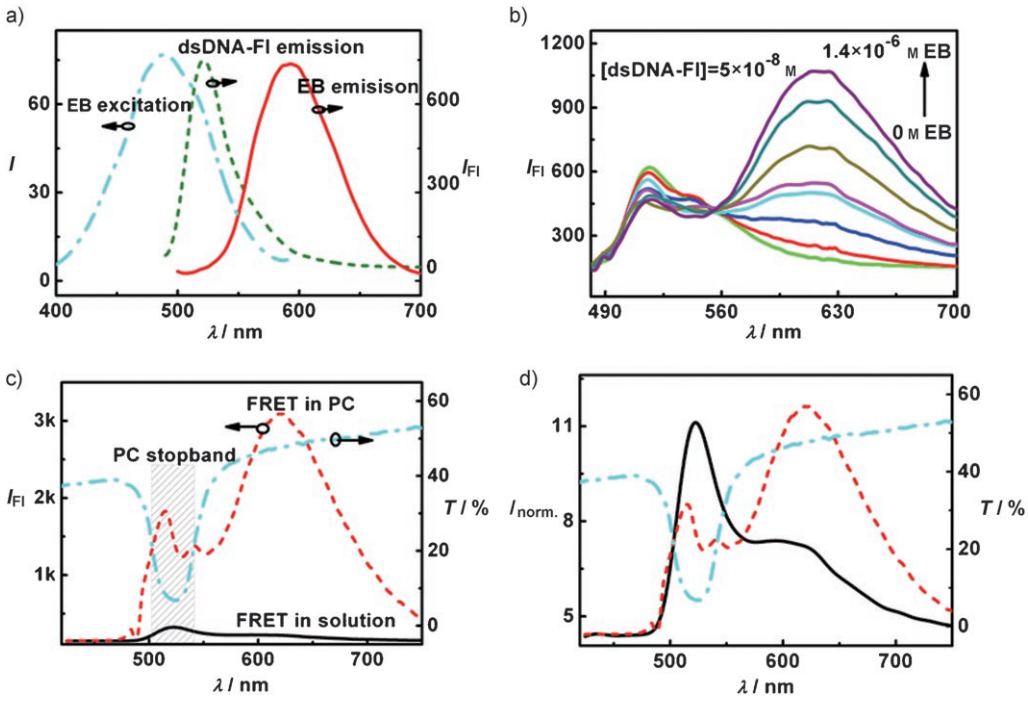

Figure 1. a) Absorption and emission spectra of EB together with the emission spectrum of EB. b) Emission spectrum of dsDNA-Fl/EB as a function of EB concentration. c) Emission spectra of dsDNA-Fl/EB; [dsDNA-Fl] = 5.0×10^{-8} M, [EB] = 1.0×10^{-6} M. d) Corresponding normalized emission spectrum (——, $I_{norm.}$) added to the spectra in PC of (c); T = transmission. Arrows in (a) and (c) indicate the relevant axis for each spectrum.

wavelength region matches well with that of the PC stop band. The results confirm that the stop band can produce strongly localized states of the donor (dsDNA-Fl) emission. There is a long lifetime for radiation injected into the cavity, and the reaction time between the donor and the acceptor will be lengthened. The donor emission can be readily generated and protected in the energy-transfer process. Thus, the energy loss of the Fl emission can be overcome and most of the energy persists to transfer to EB. Resultantly, the FRET efficiency can be remarkably enhanced, which leads to great amplification of the detection signal.

The EB emission intensity is further optimized by varying the PCs. Figure 2 shows the normalized emission spectra of dsDNA-Fl/EB in solution and in various PCs, complementary with the transmission spectra of the stop bands. According to the spectra in Figure 2a and b the FRET ratio (I_{EB}/I_{Fl}) in solution is circa 0.6. When the FRET-based detection system is immersed in the PC, the FRET emission dispersion is clearly changed. When the stop band of the PC is 520 nm (PC I), the ratio becomes 1.76, nearly three times greater than that in solution. At $\lambda_{stop\ band} = 550$ nm (PC II), the FRET ratio also increases, to 1.04. In this case, the edges of the stop band play a key role.

In the detection system, the solution enters the void of the PC, which is its low-dielectric-constant region. It has been demonstrated that the group velocity of light becomes anomalously small near the wavelength of the stop band in the PC.[10] The light waves are localized in different parts of the structure, depending on their energy. According to the electromagnetic variation theorem, the low-frequency modes mainly concentrate their energy in the high-dielectric-constant regions, whereas the high-frequency modes concentrate their energy in the low-dielectric-constant regions.[23] This fact implies that the absorbance of chromates in the low-dielectric-constant medium will be enhanced at the red edge of the stop band,[24] and their emission will be enhanced at the edges of the stop band, especially at the blue edge.[13]

For the DNA detection system within PC II ($\lambda_{stop\ band} =$ 550 nm), the donor (Fl) emission peaking at 518 nm just sits at the blue edge of the stop band, which also matches well with the acceptor (EB) emission. Therefore, both the donor emission and acceptor absorbance will be enhanced and the energy transfer can be strongly boosted. Furthermore, the maximum emission ($\lambda_{em} = 619$ nm) of the acceptor just sits at the red edge of the stop band and thus will also be enhanced to a certain level.

A comparison of the spectra in Figure 2a–c shows sufficient evidence of signal amplification by PCs, which is also indicated by additional experiments on the detection of the saturated concentration ratio of EB to dsDNA-Fl (Table 1). Here, the saturated concentration ratio refers to the ratio of the concentrations of EB and dsDNA-Fl when FRET between Fl and EB reaches a maximum at [dsDNA-Fl] $= 1.0 \times 10^{-8}$ M by addition of EB. The detection sensitivities of Fl-sensitized EB are enhanced about 9.3-fold ($\lambda_{stop\ band} =$ 520 nm) and 6.5-fold ($\lambda_{stop\ band} = 550$ nm) by the PCs, on comparing the saturated concentration ratio of EB to dsDNA-Fl. When the stop bands move away from the emission band of the donor and the absorbance band, the PCs have little influence in the FRET process because they have hardly any effect on the transition band.

In the case of PC III (Figure 2d), the EB emission is inhibited in the wavelength range of the stop band at 622 nm (see Table 1). As shown by Förster,[22] FRET refers to the

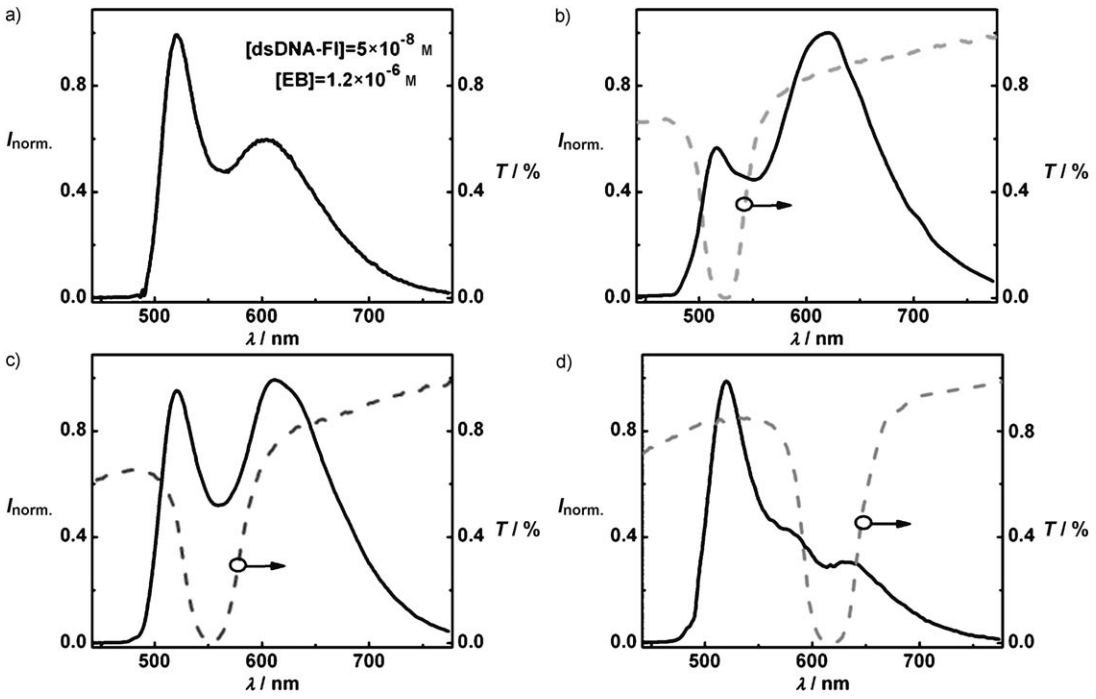

Figure 2. Emission spectra of dsDNA-Fl/EB in a) solution and b–d) various PCs complementary with the transmission spectra of the stop bands. $\lambda_{ex} = 488$ nm; [dsDNA-Fl] $= 5.0 \times 10^{-8}$ M, [EB] $= 1.2 \times 10^{-6}$ M.

Table 1: Concentration ratio of EB to dsDNA-Fl for the most intense FRET signal and the stop bands of the PCs.

	Liquid	PC I	PC II	PC III
[EB]:[dsDNA-Fl]	260	28	40	260
stop band [nm]	0	520	550	622

dipole–dipole interactions and long-range resonance energy transfer from a donor chromophore to an acceptor chromophore. It is a down-conversion process. Accordingly, there is no influence on the sensitivities of Fl-sensitized EB (Table 1) by PC III, although the emission of the acceptor (EB) is inhibited. The saturated concentration ratio is still 260, the same as that in solution (Table 1).

Within PC I, dsDNA-Fl can be detected with a sensitivity limit of approximately 13.5 fM, whereas in solution the sensitivity limit is 63.2 pM (see Figures S3a and S3b in the Supporting Information). The PC lowers the detection limit by about 468-fold. The detection limit value exceeds that of the analogous fluorophore (typically in the picomolar range; best reported ca. 600 fM) by over one order of magnitude.[25] A suitable PC is essential for the significant improvement of the sensor selectivity, the stop band of which can boost energy transfer and enhance the optical signal. PCs with sharp and complete stop bands might lead to a much-improved amplification and the detection limit could be further lowered.

Further exploration of these materials in single base-pair mismatch DNA detection reveals sharp melting transitions of the target DNA. Figure 3 shows the FRET ratios with increasing temperature for different numbers of base-pair mismatches. The fluorescence spectra are recorded at approximately every 3 K over the entire process of denaturation by gently heating the mixture from 18 °C to the full denaturation temperature. When the temperature is raised, the unwinding of dsDNA causes the release of EB and results in the disappearance of the EB emission. It can be seen that FRET can act as a good indicator of DNA melting. Deduced from the inflections of the curves, the melting temperature is 50 °C, for the fully complementary case, and drops to 46, 41, and 34 °C for one, three, and five base-pair mismatches, respectively (Figure 3b).

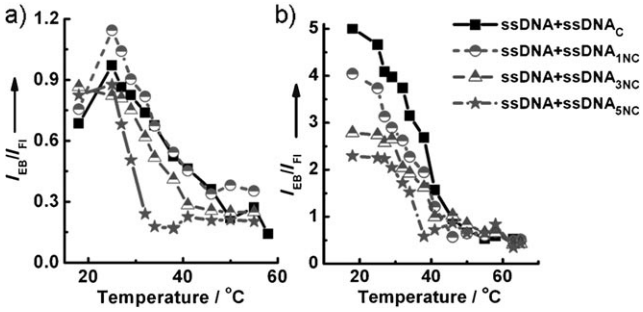

Figure 3. Change of FRET ratios with increasing temperature for different numbers of base-pair mismatches. The experiments were carried out in a) solution and b) PC II ($\lambda_{stop\ band}$ = 550 nm). [dsDNA-Fl] = 1.0×10^{-8} M; [EB] = 1.0×10^{-6} M.

In the detection system within PC II, the decay curve of the fully complementary case is distinctively different from that with base-pair mismatch. Single base-pair mismatch can be detected by integration with DNA melting analysis. For example, at 18 °C, in the detector system within the PC, more difference between the single base-pair mismatch and the fully complementary case can be observed clearly from their FRET ratios of about 5.02 and 4.04, respectively (see Figure 3b, and Figure S4b in the Supporting Information). The single base-pair mismatch can be detected under ordinary conditions at room temperature. In contrast, mismatches of base pairs are hardly visible in the emission spectra in solution (see Figure 3a, and Figure S4a in the Supporting Information).

In conclusion, we have demonstrated a PC-based light-amplification method for DNA detection. The method can achieve ultrasensitivity up to about 13.5 fM with optimized PCs. Furthermore, the PC-assisted detection method offers a great advantage over conventional techniques for its excellent ability to discriminate single base-pair mismatches, which is of crucial importance for the diagnosis of genetically encoded diseases. The result offers a promising strategy to develop highly sensitive optical biosensors and diagnose with notable sensitivity and selectivity, and shows great potential for economic solid-state sensors in PC-based DNA assays.

Experimental Section

EB was purchased from Aldrich. The ssDNAs were purchased from Shanghai Sangon Biological Engineering Technology & Services. The sequences were as follows:

ssDNA$_P$-Fl: 5′-Fl-ATCTTGACTATGTGGGYGCTAACTC-3′
complementary ssDNA$_C$: 5′-GAGTTAGCACCCACATAGT-CAAGAT-3′
ssDNA$_{1NC}$: 5′-GAGTTAGCACACACATAGTCAAGAT-3′
ssDNA$_{3NC}$: 5′-GAGTTAGCACACACAGAGTCACGAT-3′
ssDNA$_{5NC}$: 5′-GCGTCAGCACACACATACTCTAGAT-3′

The poly(St-MMA-AA) PCs were fabricated by vertical deposition from a suspension of poly(St-MMA-AA) latex spheres with volume fraction 0.15 % at 60 °C and 60 % humidity.[26] UV/Vis spectra were acquired with a Hitachi UV-4100 spectrophotometer. Scanning electron microscopy images were obtained with a Hitachi S-4700 field-emission scanning electron microscope at 3.0 kV. The emission spectra were recorded with a spectrometer/charge-coupled device system with a continuous-wave diode laser pump source. The pump wavelength was 488 nm, the maximum absorption of Fl. The laser pulses (ca. 7 ns, 1 Hz) were obtained from a frequency-tripled Nd:YAG laser (New Wave, Tempest 300, USA) and used as the excitation source. Photoluminescence (PL) spectra were obtained by a polychromator (Spectropro 550i, Acton) equipped with a liquid-nitrogen-cooled (−113 °C) charged-coupled device camera (1340 × 400 pixels) detector (SPEC-10-400B/LN, Roper Scientific). The collected PL was focused onto the entrance slit of the polychromator. A spectral resolution of 1.2 nm was obtained with a grating of 150 grooves per mm^2 and 10-μm slit. The integration time was 100 ms.

FRET experiments of the complementary and noncomplementary samples were carried out in two ways. a) The poly(St-MMA-AA) PCs were dipped in a buffer solution of the DNA sample at room temperature, successive additions of EB were performed, and the fluorescence spectra were measured. b) The poly(St-MMA-AA) PCs were dipped in a buffer solution of EB at room temperature, successive additions of the DNA sample were performed, and the fluorescence spectra were measured. The buffer solution was

potassium phosphate–sodium hydroxide (50 mM, pH 7.40). Water was purified using a Millipore filtration system.

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