Controlling the Fluorescence Resonant Energy Transfer by Photonic Crystal Band Gap Engineering

Branko Kolaric,* Kasper Baert, Mark Van der Auweraer, Renaud A. L. Vallée, and Koen Clays*

Department of Chemistry, K.U. Leuven and the Institute of Nanoscale Physics and Chemistry (INPAC), Celestijnenlaan 200F and 200D, B-3001, Heverlee, Belgium

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The fluorescence of dye molecules embedded in a photonic crystal is known to be inhibited by the presence of a pseudo-gap acting in their emission range. Here we present the first account of the influence that an incomplete photonic band gap or pseudo-gap has on the fluorescence emission and fluorescence resonant energy transfer. By inserting synthetic, donor (D)−acceptor (A)-labeled oligonucleotide structures in self-organized colloidal photonic crystals, we were able to measure simultaneously the emission spectra and lifetimes of both donor and acceptor. Our results clearly show an inhibition of the donor emission together with an enhancement of the acceptor emission spectra, indicating improved energy transfer from donor to acceptor. These results are mainly attributed to a decrease of the number of available photonic modes for radiative decay of the donor in a photonic crystal in comparison to that of the effective homogeneous medium. The fluorescence decay parameters are also dominated by the pseudo-gap acting on the energy-transfer efficiency.

Introduction

Recently, there has been an intensive effort to develop photonic devices based on organic materials, especially DNA.[1] A DNA structure (oligonucleotide) is based on two intertwined spirals of sugar and phosphate molecules mostly linked by hydrogen bonding and electrostatic interactions between base pairs. Synthesizing and manipulating different DNA molecules by physical and chemical techniques can lead to a variety of structures at the nanoscale level.[1] Since the labeling of oligonucleotides is a well-established technology, we use such labeled structures as a tool to investigate the fluorescence resonance energy transfer between D (Cy3)−A (Cy5) pairs (Figure 1) in a photonic crystal. The oligonucleotides allow for the precise control of the distance between the donor and the acceptor. The rigidity of the double strand (ds) linker provides for a very efficient energy transfer between donor and acceptor, relatively insensitive to local fluctuations that might appear in the alternative case of dyes attached to a neutral or charged polymer backbone.[2]

Photonic crystals (PCs) are materials that do not allow propagation of light in all directions, for a given frequency range, due to the periodical change of their refractive index.[3−5] An omnidirectional, propagation-free frequency range is known as a photonic band gap (PBG).[6] It has been shown that an active material (e.g., dye) with a free space radiative transition will be unable to emit a photon, if located deep inside a full PBG, due to the formation of a photon-atom bound state.[7] Therefore, the total inhibition of fluorescence emission at frequencies inside the band gap is a strong indicator for the existence of a full PBG. However, the engineering of such a full band gap photonic crystal in

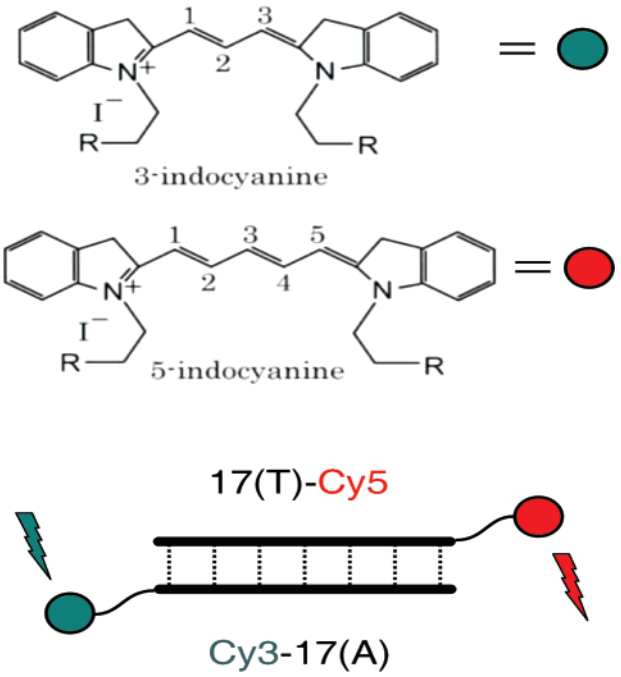

Figure 1. Schematic view of FRET between the two complementary dyes Cy3 and Cy5 attached to a double-strand backbone.

* Authors to whom correspondence should be addressed. E-mail: Branko.Kolaric@chem.kuleuven.be; Koen.Clays@fys.kuleuven.be.

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the visible region is very challenging and has not yet been realized experimentally.[8−12]

To date, only a few reports have been published considering the fluorescence resonant energy transfer (FRET), a distance-dependent dipole−dipole interaction between a donor and an acceptor, with an efficiency E_{ET} inversely proportional to the sixth power of the intermolecular separation, in nanostructured environments. In one report, the donor and acceptor dyes were statistically distributed, without control of the D−A distance in a concentrated colloidal suspension.[13] Another report of FRET in an optical microcavity suggests its importance in photonic crystals.[14] FRET plays a key role in photosynthesis[15] and its importance in the improvement of both the functionality and the efficiency of light-emitting diodes and organic lasers is increasing.[16,17] Our aim is to investigate the influence of a photonic pseudogap on the emission properties of D−A pairs embedded in a photonic crystal. Furthermore, with this approach, we extend the investigations of the radiative emission properties of single dyes embedded in a photonic crystal structure,[18−23,34] to FRET in a PC, which will allow us to develop more qualitative and quantitative descriptions of the interaction between emitters and the electromagnetic modes the emitters can couple to in a PC. This last point is crucial for the description of quantum optical phenomena in these materials.[6,9,23]

Experimental Section

To investigate and control the FRET process, two different single-strand (ss) homo-oligonucleotides (either 17 T or 17 A), one labeled with Cy3 and the other with the complementary Cy5 dye, were purchased from Jena Bioscience. These two complementary homo-oligomers were chosen to avoid the possible quenching of fluorescence emission by electron transfer that appears if a guanine base is located in the vicinity of the dye.[24] The double-strand (ds) oligonucleotide backbone was obtained by annealing.[25] For annealing and infiltration the oligonucleotides were dissolved in TRIS buffer pH = 8.5 in the presence of Mg ions.

To anneal the double-strand oligonucleotides, equal volumes of both complementary oligonucleotides (at a concentration of 100 nM dissolved in TRIS which contains 50 mM $MgCl_2$ and 50 mM NaCl, the concentration of magnesium ions was varied to optimize the annealing protocol) was mixed in a 1.5 mL microfuge tube. The tube was heated to 85−90 °C, a temperature approximately 40−50 °C higher than the melting point of the oligonucleotide. After heating, the tube was allowed to cool to room temperature (below 30 °C) on the workbench. After that the tube was stored at 4 °C until use.

Monodisperse spherical silica particles of 208 and 272 nm were synthesized by the Stöber method.[26]

The particles of 208 nm were used for the preparation of PC1 while the particles of 272 nm were used for the preparation of PC2. Photonic crystals were fabricated using the convective self-assembly approach, using the suspending power of ethanol on the silica particles, in combination with an appropriate vapor pressure for this solvent at 32 °C.[27] Both the glass substrates and the vials used for evaporation were cleaned with piranha acid ($^2/_3$ sulfuric acid, $^1/_3$ hydrogen peroxide as oxidant) prior to use. After evaporation, the resulting structures were dried at approximately 130 °C to remove any residual solvent. This resulted in photonic crystals with a face-centered cubic (fcc) or (random) hexagonal closed packing (Rhcp) crystal structures, both with a packing of 74%.

The single-strand and double-strand labeled oligonucleotides were dissolved in TRIS buffer at a 100 nM concentration. The PCs were transferred into the oligonucleotide solution with a total volume of around 500 μL. After 45 min of incubation, the slabs were dried by an argon stream, then dried at room temperature for a few hours, stored overnight at 4 °C, and measured the following day.

The extinction spectra of photonic crystals were measured using a Perkin-Elmer Lambda 900 UV−Vis NIR spectrophotometer. The ranges were presented in Figure 3a (435−480 nm for PC1; 555−640 nm for PC2). All extinction spectra were taken at normal incidence. Fluorescence emission spectra of ss-Cy3 and ds-Cy3-Cy5 dissolved in buffer were measured using a FluoroMax-3 (SPEX Instruments, Edison, NJ).

Fluorescence experiments were performed with an inverted confocal scanning optical microscope (Olympus IX70) and an excitation at 543 nm (8 MHz, 1.2 ps fwhm) from the frequency doubled output of an optical parametric oscillator (GWU) pumped by a Ti:Sapphire laser (Tsunami, Spectra Physics). The excitation light was rendered circularly polarized by a Berek compensator, to avoid the excitation polarization that was perpendicular to the transition dipole moments of the dyes which are randomly oriented in the sample. The excitation light was then directed into the inverted microscope and focused onto the sample through an oil immersion objective (1.3 NA, 100×, Olympus). The photonic crystals were oriented with the (111) direction parallel to the *z*-axis

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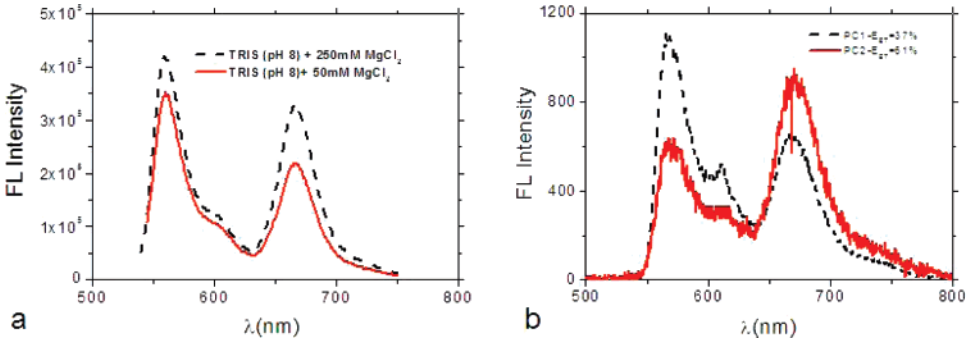

Figure 2. (a) Fluorescence of double-strand Cy3-Cy5 oligonucleotides. The strand concentration was 0.1 μM. The oligonucleotide was excited at 535 nm and the corresponding emission was recorded. (b) Fluorescence spectra of energy transfer between dyes Cy3 and Cy5 attached to a ds oligonucleotide backbone embedded within photonic crystals PC1 and PC2, respectively, measured using a confocal microscope.

of the microscope. Even though the oligonucleotides could be situated on the top of the crystal, it is reasonable to assume that the dye-labeled oligonucleotides present on the top of the crystal did not affect our results as in our detection scheme neither the excitation light nor the emission light collected passed through the top of the crystal. The fluorescence was collected through the same objective, split with a nonpolarizing beam splitter (50:50), and one path was focused into a polychromator (Spectra Pro 150 Acton Research Corporation) coupled to a back-illuminated liquid nitrogen cooled charge-coupled device (CCD) camera (LN/CCD-1340SB, Princeton Instruments) to record fluorescence spectra with a resolution down to 1 nm. In the other path, the fluorescence was again split with a dichroic mirror (Chroma 630dcxr), filtered with one bandpass filter in each path (Chroma HQ580/70 and HQ670/50) to separate the emission originating from Cy3 and Cy5, respectively, and then focused onto two avalanche photodiodes (SPCMAQ- 14, EG & G Electro Optics). The time-resolved data were collected with a TCSPC card (SPC 630, Becker & Hickl) used in the FIFO (First In First Out) mode (256 channels). To summarize, first the emission light was split in a component that went to the CCD camera to measure the fluorescence spectra; the second part was again split to record the fluorescence decay from the donor (Cy3) in one channel and from the acceptor (Cy5) in the second channel. The nonexponential decay curves were fitted with a continuous distribution of decay rates as described in detail elsewhere.[33,34]

Results

Our experimental results show the influence of a PC, with a pseudo-gap acting in the range of the spectral emission of the donor (Cy3), on the fluorescence emission of this donor covalently bound to either a single-strand oligonucleotide (ss-Cy3) or a double-strand oligonucleotide (ds-Cy3-Cy5) and on the FRET between the donor (Cy3) and acceptor (Cy5) of the labeled double-strand (ds-Cy3-Cy5). The labeled strands were infiltrated in photonic crystals with a pseudo-gap (i) outside the spectral range of the donor emission (PC1: reference) and (ii) in the spectral range of the donor emission (PC2: sample), to investigate their influence on both the fluorescence and the fluorescence energy transfer.

Figure 2a shows the fluorescence emission spectra of the annealed ds-Cy3-Cy5 labeled oligonucleotides. To optimize the annealing protocol, different salt concentrations were used.[28,29] Only conditions which were used for infiltration were shown. The highest emission peak corresponds to the donor emission (Cy3) at 562 nm and the second peak to the acceptor emission (Cy5) at 667 nm. With use of eq 1, the energy-transfer efficiency E_{ET} was determined. $E_{ET} = 38\%$ and $E_{ET} = 45\%$ respectively for a concentration of 50 and 250 mM $MgCl_2$ in a TRIS (2-amino-2-hydroxymethyl-1,3-propanediol) buffer

$$E_{ET} = 1 - \frac{I_d}{I_d + I_a} \quad (1)$$

where I_d is the intensity of the donor emission and I_a is the intensity of the acceptor emission.[2,30]

After determination of the best conditions for the annealing, ss-Cy3 and ds-Cy3-Cy5 oligonucleotides were infiltrated in a photonic crystal. Figure 3 (top) shows the extinction spectra of both the PC1 (reference) and the PC2 (sample) photonic crystals. As expected, the pseudo-gap of PC1 is outside the range of emission of the donor (562 nm, Figure 2) and the pseudo-gap of PC2 is inside this range. Furthermore, Figure 3b clearly shows that the presence of ss-Cy3 at micromolar concentrations does not significantly affect the extinction spectra and thus the photonic properties of the crystals. The observed, small blue shift is a consequence of the drying of the crystal and is not connected to the infiltration procedure. The fluorescence emission spectra were recorded to determine the influence of the pseudo-gap on the fluorescence emission of the dyes. The fluorescence emission of Cy3 embedded in PC2 divided by the Cy3 emission from PC1 (after normalization at long wavelength) is shown in Figure 3c. The presence of the photonic pseudo-gap in the spectral range of the emission of Cy3 causes a decrease in the fluorescence emission compared with the emission from the dye embedded in the reference photonic crystal (PC1). A band diagram for photonic crystals similar to the ones used in our experiments can be found in Pallavidino et al.[31]

Figure 2b shows the fluorescence energy transfer between the donor (Cy3) and the acceptor (Cy5) attached to the double-strand oligonucleotide backbone and embedded within photonic crystals PC1 (reference, black curve) and PC2 (sample, red curve). The presence of the pseudo-gap in the spectral region of the donor emission (PC2) causes a decrease

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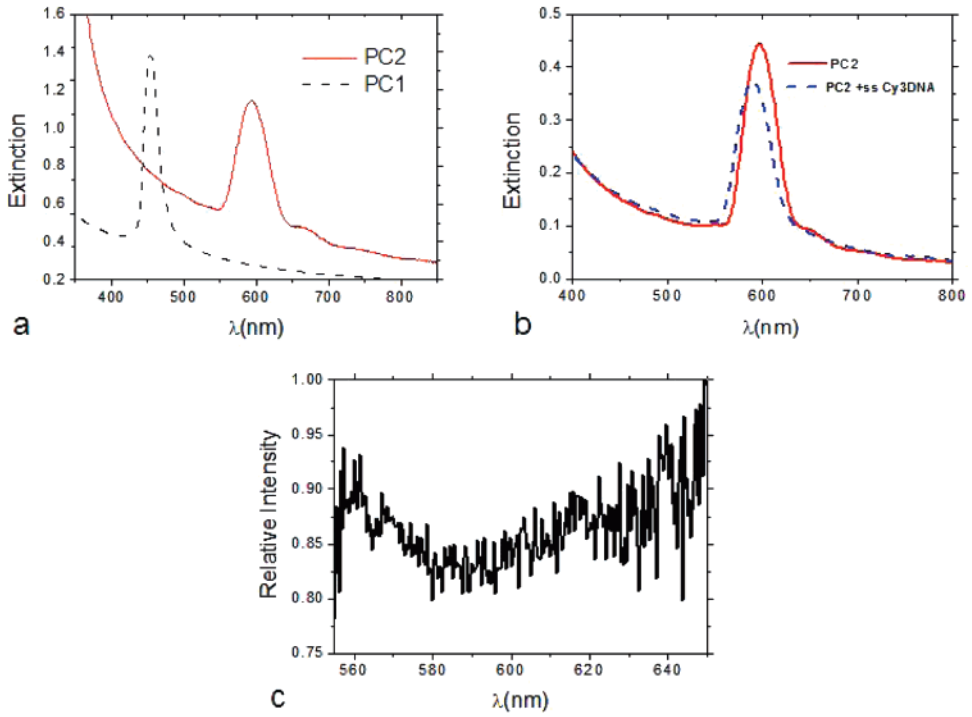

Figure 3. Extinction spectra of the photonic crystal with a pseudo-gap outside the emission region of the dye (PC1) and of the photonic crystal with a pseudo-gap in the range of Cy3 emission (black), (a) without ss-Cy3, (b) after infiltration with ss-Cy3 (0.1 μM) in PC2, and (c) the fluorescence emission of ss-Cy3 embedded in PC2 divided by the Cy3 emission from PC1 (after normalization at longer wavelength).

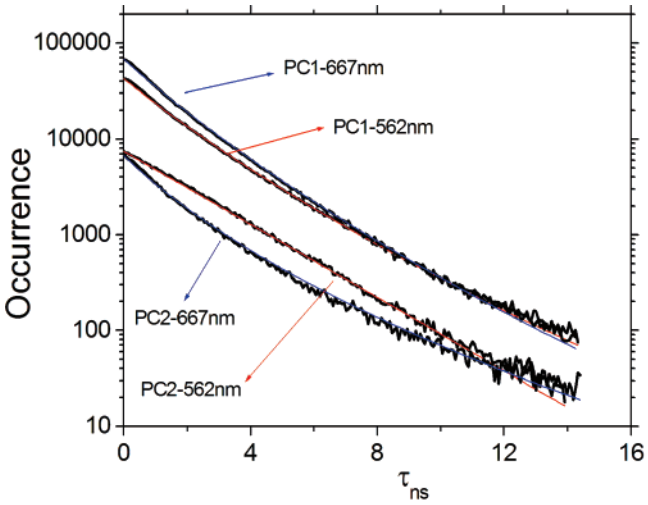

Figure 4. Measured and fitted fluorescence decay of the dyes attached to a ds oligonucleotide and embedded within PC1 and PC2 respectively measured at 562 and 667 nm.

of the spontaneous emission of the donor and an increase of the spontaneous emission of the acceptor. The FRET efficiency (eq 1) is $E_{ET} = 37\%$ for D−A pairs embedded in PC1 (reference) and $E_{ET} = 61\%$ for PC2 (sample).

To get more detailed investigations of the influence exerted by the pseudo-gap on the fluorescence emission of Cy3 and the energy transfer between Cy3 and Cy5, fluorescence decay profiles of ss-Cy3 and ds-Cy3-Cy5 oligonucleotides embedded in photonic crystals PC1 and PC2 were measured and analyzed. A typical example of the fitted decay profiles can be found in Figure 4. Please note that in phosphate buffer (PBS) the pure dyes exhibit single exponential fluorescence decays (decay time of Cy3, 0.3 ns; Cy5, 1.5 ns).[32] The presence of a DNA backbone causes an increase of the decay times of the dyes due to a partial stabilization of the excited

Table 1. Decay of ss-Cy3 Embedded in a Photonic Crystal Was Recorded at 562 nm, Taken from Different Spots in the Crystal[a]

PC1 (τ,Γ_{mf})	PC2 (τ,Γ_{mf})	$\Delta\gamma$ PC1 562	$\Delta\gamma$ PC2 562
2.4 (0.42)	2.8 (0.36)	0.72	0.32
2.4 (0.42)	2.8 (0.36)	0.72	0.37
2.3 (0.43)	2.6 (0.38)	0.68	0.39

[a] τ, average decay time; Γ_{mf}, most frequent rate; $\Delta\gamma$, distribution width.

states of the dyes by the hydrophobic part of the oligonucleotide backbone.[2,30,32] Only bases very close to the dyes can have a significant influence on their excited states. Accordingly, the decay times of Cy3 and Cy5 vary for different oligonucleotides and oligonucleotide structures due to the different direct environment of the dyes.[2,36] In the case of Cy3 and Cy5, the presence of the backbone causes the appearance of multiexponential decay profiles.[29,35,32]

By infiltration of the labeled oligonucleotides in a PC, the decay profiles of the dyes are drastically changed, so that it was no longer possible to fit these decays with a single, double or stretched (KWW) exponential function. According to an approach developed by van Driel and others for emitters in a photonic crystal,[33,34] we used a nonexponential fitting procedure with a continuous distribution of decay rates. The idea behind this model is that the (emission transition dipole moment of the) emitters are spatially and orientationally distributed in the photonic crystal. As such, each emitter probes a different local environment with a different density of optical modes to which it can couple to, leading to a distribution of decay rates. The results from the fitting, i.e., the most frequent decay rates (Γ_{mf}) and the widths ($\Delta\gamma$) of the continuous distributions of decay rates, are shown in Tables 1 and 2. These two parameters are fully described

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Table 2. Decays of ds-Cy3-Cy5 Embedded in a Photonic Crystal Were Recorded at 562 and 667 nm, Respectively, Taken from Different Samples[a]

Cy3				Cy5			
PC1 (τ,Γ_{mf}) 562	PC2 (τ,Γ_{mf}) 562	$\Delta\gamma$ PC1 562	$\Delta\gamma$ PC2 562	PC1 (τ,Γ_{mf}) 667	PC2 (τ,Γ_{mf}) 667	$\Delta\gamma$ PC1 667	$\Delta\gamma$ PC2 667
2.4 (0.42)	2.4 (0.42)	0.017	0.017	2.5 (0.40)	1.9 (0.52)	0.28	0.78
2.5 (0.40)	2.4 (0.42)	0.21	0.03	2.5 (0.40)	2.2 (0.46)	0.24	0.43
2.4 (0.42)	2.2 (0.46)	0.17	0.017	2.4 (0.42)	2.0 (0.50)	0.26	0.32
2.3 (0.44)	2.5 (0.40)	0.018	0.018	2.1 (0.48)	1.9 (0.52)	0.4	0.49
2.4 (0.42)	2.5 (0.40)	0.017	0.016	2.0 (0.50)	2.2 (0.46)	0.30	0.46
2.2 (0.46)	2.6 (0.38)	0.018	0.016	2.0 (0.50)	2.2 (0.46)	0.45	0.46
2.3 (0.44)	2.2 (0.46)	0.016	0.015	1.8 (0.54)	1.8 (0.56)	0.54	0.66
2.4 (0.42)	2.4 (0.42)	0.018	0.018	1.9 (0.52)	1.8 (0.54)	0.45	0.53

[a] τ, average decay time; Γ_{mf}, most frequent rate; $\Delta\gamma$, distribution width.

elsewhere.[33,34] The reciprocal value of the most frequent rate $\tau = \Gamma^{-1}$ represents the average decay time of the dye.

Table 1 shows that the infiltration of the ss-Cy3 oligonucleotides in photonic crystals (at void−silica spheres interfaces) causes an increase of the decay time in comparison with the solution measurements.[2,32] This increase can be explained by a geometrical confinement effect which suppresses internal conversion[35] and by the adsorption of the dye on the silica sphere surface which results in a different environment around the dye in comparison with a buffer solution. Furthermore, the presence of the pseudo-gap (PC2) in the emission range of Cy3 causes an increase of the decay time of approximately 0.3 ns in comparison to ss-Cy3 embedded in PC1. Also, $\Delta\gamma$, the width of the distribution of decay rates,[33] decreases by 45% when the dye is embedded in PC2 rather than in PC1.

Table 2 shows that the Γ_{mf} and $\Delta\gamma$ of the continuous distributions of decay rates for Cy3 attached to a ds oligonucleotide are very similar in the PC1 and PC2 photonic crystals. In this case, the energy transfer occurs between the donor (Cy3) and the acceptor (Cy5). The observed width $\Delta\gamma$ of the distributions of Cy3 becomes very narrow, indicating an almost single exponential behavior. For all samples the widths $\Delta\gamma$ of the continuous decay rate distributions for Cy5 in PC2 are higher than for those embedded in PC1.

Discussion

In the previous section, we mentioned that different buffers and concentrations of salts were used to optimize the annealing procedure, i.e., to get a more rigid and compact ds oligonucleotide, which would then optimize the efficiency of the fluorescence energy transfer.[2,36] The presence of salts is crucial for stabilizing the electrostatic interactions between the oligonucleotide strands.[29] The stabilization must be strong enough to overcome interactions between the oligonucleotides and the surface of the colloidal spheres that compose the photonic crystal. These interactions occur during the infiltration of the labeled oligonucleotides in the photonic crystal. If the stabilization is not strong enough, the ds oligonucleotides start to unfold, leading to an increased distance between D−A pairs and the FRET process is suppressed. Figure 2a clearly exhibits that this goal has been reached with an optimized concentration of 250 mM $MgCl_2$ in a TRIS buffer, causing a E_{ET} of 45%.

Recently, it was shown that inside an opal the distribution of dye molecules is not uniform and that near the edges of the crystal it fills voids and channels connecting those voids, whereas in the center dye molecules are primarily found in the voids.[37] The dye aggregation within the voids is concentration-dependent (and is observed at higher concentrations) and depends strongly on solvent, ionic strength, and temperature. Taking into account that we used a labeled oligonucleotide at a concentration of 100 nM, the aggregation within voids should be excluded since aggregation was not observed in the emission spectra. The observed spectra correspond nicely to the spectra of dyes in solution. Considering that both PCs are made from silica spheres, electrostatic and van der Waals interactions, which control the interactions between oligonucleotides and silica, are the same for both PC1 and PC2 due to the identical chemical structure of the silica spheres used to make both PC1 and PC2. The observed differences in energy transfer between PC1 and PC2 can only be attributed to the photonic confinement and the presence of a band gap in PC2 where the photonic band gap position corresponds to the range of the dye emission.

Figure 3 clearly shows that we succeeded in engineering a photonic crystal PC2 with a pseudo-gap in the spectral range of 561−615 nm that covers a substantial part of the fluorescence emission of Cy3. Because of the low dielectric contrast between the silica particles and air, the band is not a full gap and hence the fluorescence emission was not inhibited completely in all directions in this range of frequencies: only a fluorescence emission decrease of the Cy3 dye in PC2 as compared to PC1 was observed. This suppression of emission was expected due to the decrease[38,19,34] of the number of available optical modes in the pseudo-gap for PC2.

In the case of energy transfer between the D−A pairs attached to the ds oligonucleotide backbone, the presence of a pseudo-gap (PC2) in the spectral range of the donor (Cy3) causes a decrease of donor emission and an increase in acceptor emission, with respect to the reference sample (PC1) (Figure 2b). Inside the PC2, the Cy3 spontaneous emission is inhibited. A redistribution of energy thus has to take place so that the nonradiated energy is partially or totally transferred to the acceptor Cy5, leading to an enhancement of the energy transfer with respect to the reference sample (PC1). As such, an increased suppression of the donor emission will lead to the increased enhancement of the

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acceptor emission.[7] At this point, it is not possible to determine quantitatively which part of the energy is transferred to the acceptor and which part is dissipated to the environment. To obtain more detailed information about energy transfer in the photonic crystals, the fluorescence decays were measured using single-photon timing spectroscopy.

The decrease of the spontaneous emission rate for ss-Cy3 within PC2 in comparison to PC1 is around 15% (Table 1). This decrease is bigger than the one observed for fluorescein molecules (4%) embedded in similar colloidal crystals.[34] The decrease with respect to PC1 of both spontaneous emission rates and distribution widths correlates well with the observed suppression of fluorescence in the emission spectra when the labeled oligonucleotide (ss-Cy3) is embedded in, and thus controlled by, the pseudo-gap of PC2.[33,34,39]

In the case of the ds-Cy3-Cy5 structure (Table 2), the vanishing of the difference between the Cy3 decay times in PC1 compared to PC2, which was seen for the ss-Cy3 case (Table 1), is governed by the aperture of a nonradiative channel where energy is transferred to the acceptor. The fact that these decay times are very similar in both samples indicates that the energy-transfer process is taking place at a slightly faster rate than the radiative rate irrespective of the control by the pseudo-gap. The same argument explains the (re) appearance of a single exponential decay ($\Delta\gamma \sim 0.02$): as the energy-transfer rate is the dominant rate, it tends to homogenize the observed rates as well.

In the case of the acceptor Cy5, the average decay rates are similar for all samples whether the ds oligonucleotides are embedded in PC1 or in PC2. It is important to stress that the width of the continuous rate distributions of Cy5 in PC2 are higher than the ones for Cy5 embedded in PC1. As the energy transfer is enhanced in PC2, more emitters, situated at different locations with different orientations, are excited, which leads to a higher $\Delta\gamma$. This increase of $\Delta\gamma$ correlates nicely with the enhancement of energy transfer in PC2 observed in the steady-state measurements.

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Conclusion

We have shown that photonic crystal band gap engineering of a pseudo-gap can be used to modify the FRET between a D−A pair. Steady-state spectra of the donor and acceptor dyes embedded in photonic crystals clearly show that a correctly engineered pseudo-gap causes a decrease in donor emission and an increase in acceptor emission. The suppression of the donor emission and enhancement of the energy transfer is explained by a lack of available modes for radiative decay of the donor in the photonic crystal compared with the effective homogeneous medium. The influence of the photonic crystal on energy transfer is confirmed by the decay time of the dyes. The photonic crystal modifies the absolute value of the decay time of the dye and causes nonexponential fluorescence decays. The most frequent decay rate Γ_{mf} and the distribution width $\Delta\gamma$ for a Cy3 labeled ss oligonucleotide decrease when it is embedded in a PC that suppresses Cy3 emission as compared to a PC that does not. In the case of FRET in a photonic crystal, differences in the decay rates between Cy3 embedded in PC1 and PC2 disappear due to the opening of a nonradiative channel which transfers energy from the donor to the acceptor.

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