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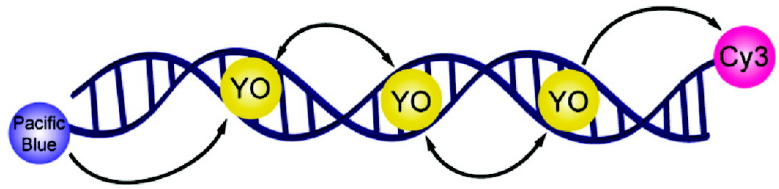

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Self-Assembled DNA Photonic Wire for Long-Range Energy Transfer

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Abstract: DNA is a promising material for use in nanotechnology; the persistence length of double stranded DNA gives it a rigid structure in the several-nanometer regime, and its four letter alphabet enables addressability. We present the construction of a self-assembled DNA-based photonic wire capable of transporting excitation energy over a distance of more than 20 nm. The wire utilizes DNA as a scaffold for a chromophore with overlapping absorption and emission bands enabling fluorescence resonance energy transfer (FRET) between pairs of chromophores leading to sequential transfer of the excitation energy along the wire. This allows for the creation of a self-assembled photonic wire using straightforward construction and, in addition, allows for a large span in wire lengths without changing the basic components. The intercalating chromophore, YO, is chosen for its homotransfer capability enabling effective diffusive energy migration along the wire without loss in energy. In contrast to heterotransfer, i.e., multistep cascade FRET, where each step renders a photon with less energy than in the previous step, homotransfer preserves the energy in each step. By using injector and detector chromophores at opposite ends of the wire, directionality of the wire is achieved. The efficiency of the wire constructs is examined by steady-state and time-resolved fluorescence measurements and the energy transfer process is simulated using a Markov chain model. We show that it is possible to create two component DNA-based photonic wires capable of long-range energy transfer using a straightforward self-assembly approach.

Introduction

Development in the field of nanotechnology has seen a recent focus toward bottom-up approaches as a construction strategy. Using self-assembly as a key technique has enabled the creation of nanoscale constructs with increasingly finer structure. DNA has been one of the building materials used in a wide variety of arrangements, ranging from planar assemblies[1−3] to tubes[4,5] and three-dimensional structures.[6−8] By using the four letter coding language of DNA, it is possible to create addressable structures, allowing for postformation functionalization of the DNA network.[9,10] For these structures to be used in nanoscale applications using multiple communicating components, there must be ways to convey information with molecular precision. The light harvesting complexes in photosynthetic organisms are some of nature's most remarkable examples of molecular engineering. Here, solar energy is transferred from light harvesting complexes in several steps with high precision to the reaction center.[11−13] These antenna systems transport the excitation energy through a large number of both strong and weak interactions. One way that energy is transported is through (fluorescence) resonance energy transfer (FRET), which involves the dipole−dipole interaction between chromophores with overlapping emission and absorption bands and is described by the Förster theory.[14] The transfer efficiency is strongly dependent on the distance between the donor and acceptor chromophores; thus, FRET can be used to transfer energy rather efficiently in one step up to ∼80 Å. By utilizing multistep energy transfer, distances over the limitations of direct transfer can be bridged. To achieve this, the constituent chromophores must be carefully positioned so that the energy transfer is efficient in each step, resulting in an efficient long-range energy transfer.[15−17] The DNA molecule can serve as a scaffold for positioning a series of chromophores with overlapping absorption and emission bands. Previously, multistep FRET has been achieved using

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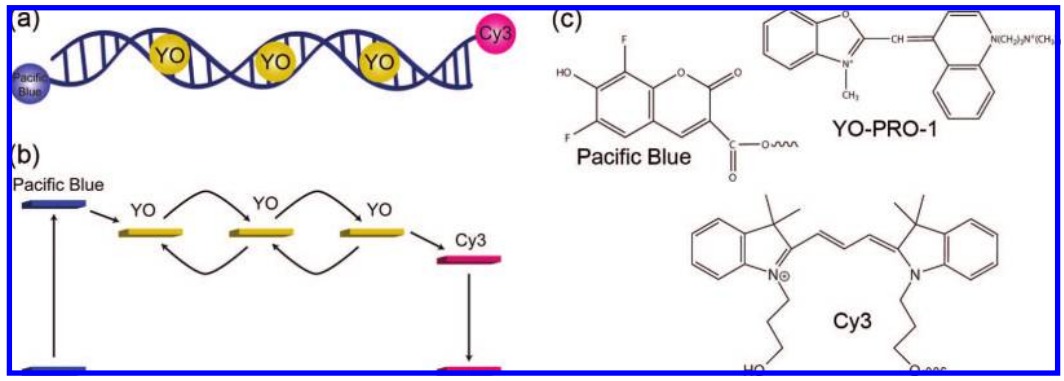

Figure 1. (a) Schematic representation of the multichromophoric DNA wire with attached Pacific Blue (injector) and Cy3 (reporter) and intercalated YO. (b) Corresponding schematic energy level diagram, showing the diffusive energy migration in the YO section of the wire. (c) Structures of Pacific Blue, YO-PRO-1, and Cy3, the three chromophores used in the construction of the photonic wire.

several different chromophores, covalently attached to the DNA, arranged in a cascade-like manner going downhill in energy from the initial donor to the final acceptor.[18−21] This gives a high directionality, but at the same time, since each step leads to a red-shift in the energy of the emission there is a significant energy loss for the transferred photon. A variation of this is the use of an array of covalently attached, identical chromophores capable of homotransfer as a relay between two end chromophores.[22−24] In this approach, excitation energy is conveyed in a diffusive manner, giving no loss in energy associated with a red-shift in emission but with the drawback of decreased directionality. To be able to utilize the energy transported by the wire to, e.g., drive chemical reactions, it is important to minimize the energy loss associated with the transport along the wire.

Here, we focus on creating a self-assembled DNA-based photonic wire. This is achieved by using an intercalating chromophore, YO, as an energy mediator between covalently attached donor (injector) and acceptor (reporter) chromophores at opposite ends of the DNA strand (Figure 1). The homotransfer capabilities of YO allow for end-to-end energy transfer with minimal energy losses due to red-shift of the transferred energy. Because the interaction between the intercalating molecule and the DNA strand is of a noncovalent nature, it is possible to add the intercalator after the two DNA strands have been hybridized, converting the scaffold into an energy-conducting wire. This means that any two complementary DNA strands can be converted into a photonic wire by the simple addition of YO. The length limitation of this setup is determined by the energy hopping efficiency between the intercalated relay molecules.

Our results show that the energy transfer efficiency is strongly dependent on strand length and chromophore density. Nevertheless, energy transfer is clearly demonstrated to be rather efficient in wires with lengths up to ∼20 nm. This clearly illustrates that it is possible to create a photonic wire capable of long-range transfer of excitation energy using intercalated chromophores.

Materials and Methods

Sample Preparation. Photonic wires of the two different lengths were created using deoxyoligonucleotides labeled with fluorescent probes. One 50-mer sequence, 5′−TCA GTG ATT AGT CAT CTC GTA TTC TTG CTA TCT CGA TAT TGA GTT GCA GA−3′ (A), and one 20-mer sequence, 5′−TCA GTG ATT AGT CAT GCA GA−3′ (B), were used together with their complementary strands (Ac and Bc, respectively). The sequences were designed to avoid fraying ends and formation of unwanted secondary structures. Strands A and B were purchased 5′-labeled with Pacific Blue (3-carboxy-6,8-difluoro-7-hydroxycoumarin) from Eurogenetec and the complementary Ac and Bc strands, 5′-labeled with Cy3 2-[(1E,3E)-3-[1,3-dihydro-1-(3-hydroxypropyl)-3,3-dimethyl-2H-indol-2-ylidene]-1-propen-1-yl]-1-(3-hydroxypropyl)-3,3-dimethyl-3H-indolium, were a kind gift from Prof. Tom Brown. Equimolar amounts of the strands (A and Ac, B and Bc) were mixed in sodium phosphate buffer (50 mM Na^+, pH 7.5) at room temperature to a concentration of 0.5 μM double stranded DNA. Annealing was performed by heating the samples to 80 °C for 10 min, followed by a slow cooling to room temperature (∼6 h). After the strands were annealed, the intercalating relay molecule YO-PRO-1 (4-[(3-methyl-2(3H)-benzoxazolylidene)methyl]-1-[3-(trimethylammonio)-propyl], Molecular Probes) was added from a 100 μM (in phosphate buffer) stock solution to give a YO/base pair ratio spanning from 0 to 0.35 in the assembled 20-mer wire and to 0.4 in the 50-mer wire.

Steady State Absorption and Fluorescence Measurements. Steady state absorption and fluorescence measurements were performed using a Cary 4000 UV−vis spectrophotometer and a SPEX fluorolog 3 fluorimeter, respectively. The measured sample was placed in a 50 μL quartz cell with 3 mm path length and kept at 22 °C during the measurement. Corrected emission spectra were collected using an excitation wavelength of 380 nm.

Time-Resolved Fluorescence. The fluorescence decays were measured using time correlated single photon counting (TCSPC). A Tsunami Ti-Sapphire laser (Spectra Physics) was used to generate a 760 nm output pulse with a width of 1−2 ps. Using a pulse selector (Model 3980, Spectra Physics), a 4 MHz repetition rate was generated. The generated light was then passed through a

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frequency doubler (GWU-FHG, Spectra Physics) to obtain excitation light at 380 nm. Emitted light was detected using a R3809U-50 MCP photomultiplier (Hamamatsu) together with a SPC-300 TCSPC device (Becker & Hickl GmbH). A more detailed description of the instrumental setup is given elsewhere.[25] The instrument response function (IRF) was measured using a scattering solution (silica sol) and the fwhm was typically about 50 ps. The measured fluorescence decay, $F(t)$ is the convolution of the IRF with the intensity decay function, $I(t)$. The intensity decay function was fitted using a sum of exponentials, and the goodness of the fit was judged by the reduced χ^2 value and visual inspection of the weighted residuals.

Data Analysis. In the steady state emission spectrum, the spectra of the different chromophores will substantially overlap. To extract the contribution from each single component, least-squares fitting of the spectrum was performed using standard spectra for each individual chromophore in the measured wavelength interval:

$$\mathrm{PYC}(\lambda) = pP(\lambda) + yY(\lambda) + cC(\lambda) \quad (1)$$

where PYC is the spectrum of the complete wire with Pacific Blue (P), YO (Y), and Cy3 (C). P, Y, and C are the measured standard spectra of the constituents and p, y, and c are their individual weights. The least-squares fitting procedure was performed both for the complete wire containing Pacific Blue, YO and Cy3 and for the one without Pacific Blue. The contribution to the Cy3 emission from directly excited YO, and subsequent FRET to Cy3, was subtracted from extracted emission for the complete wire to yield only the Cy3 emission resulting from transfer from Pacific Blue. To estimate the end-to-end transfer efficiency of the wires, the number of excited Cy3 molecules is compared with the number of initially excited Pacific Blue molecules. By dividing the extracted Cy3 emission with the fluorescence quantum yield for Cy3 (0.16 in double-stranded DNA[26]) a measure of the total excitation energy transferred from Pacific Blue is obtained. In the same manner, the unquenched Pacific Blue emission is divided by the Pacific Blue quantum yield (0.75[27]) to give the number of initially excited Pacific Blue molecules. This is described mathematically in eq 2,

$$E = \frac{(F_{\mathrm{CP}} - F_{\mathrm{C}})/Q_{\mathrm{C}}}{F_{\mathrm{P}}/Q_{\mathrm{P}}} \quad (2)$$

where F_{CP} an F_{C} are the integrated Cy3 emission spectra on the wavenumber scale with excitation at 380 nm from the wire with and without Pacific Blue, respectively. F_{C} gives a measure of the Cy3 emission coming from directly excited YO and is subtracted from the Cy3 emission of the complete wire. F_{P} is the integrated Pacific Blue emission from a corresponding wire containing only Pacific Blue, and Q_{C} and Q_{P} are the quantum yields of Cy3 and Pacific Blue, respectively. The spectra were collected under equivalent conditions with the same chromophore concentrations. The model used to calculate the end-to-end transfer efficiency assumes that there is no direct excitation of Cy3 at 380 nm.

Simulation of Energy Transfer. The propagation of excitation energy along the wire was simulated using a Markov chain model based on a sequence of different energy transfer probabilities. The model is of a semiempirical nature and uses the experimentally determined Förster distances of the used chromophores. A similar approach has previously been used to model the fluorescence depolarization of YO intercalated in DNA.[28] This model was modified by incorporating Pacific Blue and Cy3 as flanking chromophores in each end of the strand. To determine the end-to-end energy transfer efficiency, the excitation energy was injected at the Pacific Blue position and the output at the Cy3 position was monitored. Between Pacific Blue and Cy3, intercalated YO was randomly distributed at a given binding ratio and obeyed the nearest neighbor exclusion principle. Further details regarding the model used for the simulations can be found in the Supporting Information.

Results and Discussion

We use the three chromophores Pacific Blue, YO, and Cy3, together with double-stranded DNA, to create and characterize a photonic wire. Pacific Blue is used as an initial donor chromophore, injecting excitation energy into the wire. Cy3 acts as a terminal acceptor, offering the possibility to evaluate the wire performance by recording its emission. YO is an intercalator that inserts with high affinity between the base pairs of DNA following the nearest neighbor exclusion principle[29] and is used as an energy relay, bridging the distance between Pacific Blue and Cy3. In addition to being a strong intercalator, YO has a significant overlap between its absorption and emission bands, enabling homotransfer between different YO monomers. When added to the assembled DNA strand, YO intercalates and mediates energy transfer from one end to the other. Since YO is virtually nonfluorescent in aqueous solution, there is no contribution to the background fluorescence from unbound YO.[30] The mean interchromophore distance in the wire is modified by varying the intercalator density, i.e., increasing the relative concentration of YO.

The efficiency of the multistep FRET, and thus, the efficiency of the wire to mediate excitation energy, is evaluated by measuring the sensitized emission from Cy3 (acceptor) when exciting Pacific Blue (donor) at 380 nm. At this wavelength, there is virtually no direct excitation of Cy3, i.e., all emission from Cy3 is a result of energy transfer, either directly from Pacific Blue or relayed through YO. However, in the compiled wire, there is a substantial possibility to directly excite YO chromophores at 380 nm. Because of this, there will be Cy3 emission arising from excitation of YO and subsequent energy transfer to Cy3, i.e., sensitized emission not originating from the excitation of Pacific Blue. At each YO concentration, this effect is measured on a wire without Pacific Blue, with the corresponding concentration of YO. Moreover, the direct energy transfer from the excited Pacific Blue to Cy3 is measured on the wire without any added YO. Two different lengths of wire (oligonucleotides) are investigated, a shorter 20-mer (~7 nm) and a longer 50-mer (~17 nm).

20-mer Photonic Wire. For the shorter, 20-mer wire, the added YO concentrations range between an average of 0 and 7 YO molecules per strand, corresponding to YO/base pair (bp) ratios from 0 to 0.35. Intercalation of one YO molecule extends the length of the DNA with approximately one base pair (0.34 nm). This means that the wire length will increase from ~6.8 nm to ~9.2 nm when adding increasing amounts of YO up to a maximum of 0.35 YO/bp. Because of the diffusive energy migration in the YO part of the wire, a large number of intercalated YO molecules on each strand leads to multiple possible energy transfer pathways. In the 20-mer system, the interesting cases include the direct Pacific Blue-Cy3 transfer

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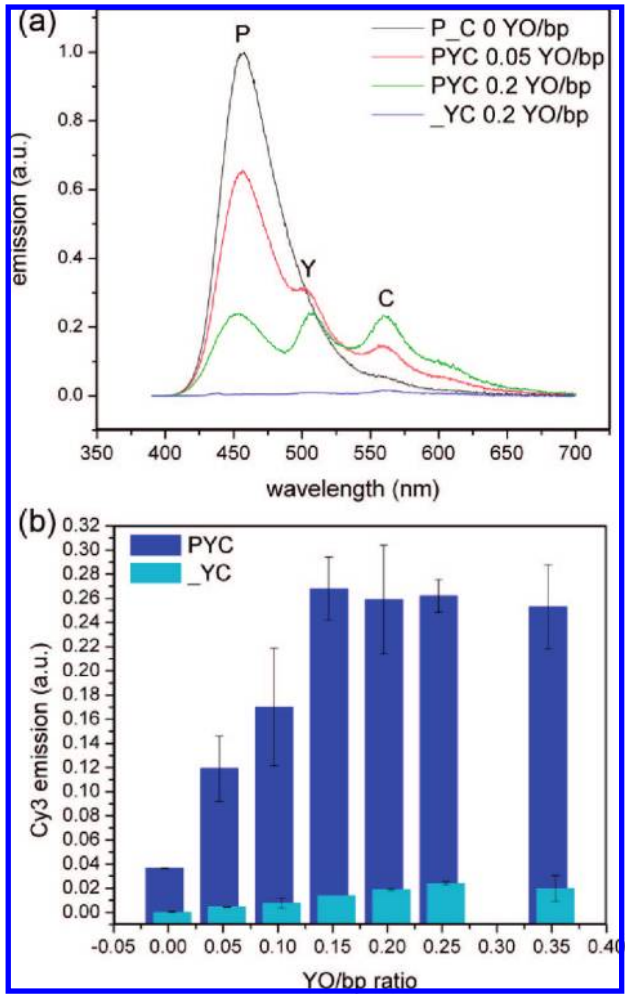

Figure 2. (a) Measured emission spectra of the 20-mer photonic wire for various YO concentrations (excited at 380 nm). Indicated are peaks for Pacific Blue (P), YO (Y), and Cy3 (C), respectively. P_C is the wire with only Pacific Blue and Cy3, _YC is the wire with YO and Cy3 and PYC is the complete wire containing all three chromophores. (b) Extracted Cy3 emission from strands with and without attached Pacific Blue. The data is the average of two separate measurements. The data extraction method used is described in the Materials and Methods section.

(0 YO/bp), the two step transfer Pacific Blue-YO-Cy3 (0.05 YO/bp, i.e., one YO/strand), and the multiple step transfers (YO/bp $\geq$0.1, i.e., YO/strand $\geq$2). Results from steady state measurements on these systems are presented in Figure 2a.

In the absence of YO, exciting the sample at 380 nm where Pacific Blue dominates the absorption spectrum results in a large peak representing the Pacific Blue emission (Figure 2a). Also visible in the spectrum is a weak emission from Cy3, showing the presence of direct transfer from Pacific Blue to Cy3 (since direct excitation of Cy3 at 380 nm gives no measurable emission, data not shown). When going from the direct transfer case to the two-step transfer (YO/bp ratio = 0.05, 1 YO/strand, Figure 2a), a significant increase in Cy3 emission is observed. However, there is still a substantial degree of Pacific Blue emission left, indicating that the efficiency of energy injection into the wire can be improved. At low YO concentration, there may be a substantial amount of wires with no intercalated YO, and thus with a low energy transfer efficiency. Therefore, the YO concentration needs to be increased to achieve the overall maximum transfer efficiency even though a two step transfer is sufficient to bridge the Pacific Blue to Cy3 distance for the short 20-mer wire. Further increasing the YO concentration results in an increase of Cy3 emission and a decrease of the Pacific Blue emission (Figure 2a). Also shown in Figure 2a is a spectrum of a sample without Pacific Blue at a YO/bp ratio of 0.2. This spectrum demonstrates that the Cy3 emission emanating from direct excitation of YO and subsequent energy transfer to Cy3 is very low and not on the level of the emission in the presence of Pacific Blue.

The Cy3 component in the emission spectra is extracted from the measured spectra by least-squares curve fitting according to eq 1. The extracted Cy3 emission intensities for the complete 20-mer wire (PYC) and for the wire without Pacific Blue are presented in Figure 2b. For the complete wire, the Cy3 intensity shows a steep initial increase when YO is added, whereas for the wire without Pacific Blue, there is only a slight increase in the Cy3 emission. This shows that the observed Cy3 fluorescence for the complete wire is mainly a product of multistep energy transfer going from Pacific Blue through the intercalator YO to reach Cy3. A leveling off in Cy3 intensity is reached at YO/bp larger than 0.15, which corresponds to an average of three YO/strand. Although a higher YO density means shorter interchromophore distances, which in turn should result in a more effective energy transfer, the Cy3 emission remains constant at YO/bp ratios >0.15. The reasons for this can be manifold, for example, as more YO is added to the wire, the number of steps that the excitation energy will take before arriving at the terminal Cy3 will increase. Since in each step there are radiative and nonradiative deactivation pathways competing with the energy transfer, increasing the number of FRET steps will consequently increase the number of ways that the excitation energy can escape the system before arriving at the terminal Cy3. Furthermore, previous studies have shown that at YO concentrations above 0.2/bp, other binding modes than intercalation will start to have a substantial contribution to the YO-DNA interaction, and these externally bound YO molecules might function as energy traps from which further energy hopping is not possible.[29] In addition, the interaction between two closely positioned YO monomers can be so strong that localized energy wells are created. We have observed a change in the shape of the absorption band of YO when going to high concentration, which indicates the presence of different binding modes and/or exciton coupling between closely positioned YO monomers. These properties and processes will affect the propagation of excitation energy along the wire and may contribute to the leveling out of the Cy3 emission observed for high YO concentrations. Using eq 2, the end-to-end transfer efficiency is estimated to over 80% for YO/bp ratios above 0.15, which can be compared to the direct (Pacific Blue-Cy3) efficiency of ~13%. The inhibiting effect from alternative binding modes of YO is not strong enough to give a substantial decrease in end-to-end transfer efficiency. This indicates that the majority of the transferred energy only passes one or two YO molecules before reaching Cy3. Because of this, there is little diffusive character in the energy transfer of the 20-mer, and thus, only little effect of the inhibiting binding modes on the overall efficiency is seen.

50-mer Photonic Wire. The experiments with the 20-mer strand show that it is possible to create a photonic wire with the use of an intercalating molecule as a relay, bridging the distance between the energy injector and the terminal acceptor chromophores. To create a wire that could be incorporated into structures of sizes obtainable from top-down lithographic techniques, it is necessary to go to greater strand lengths. A

50-mer DNA strand without added YO corresponds to a wire length of ∼17 nm, and when YO is added the length ranges from ∼18 nm to ∼24 nm for a YO/bp ratio from 0.05 to 0.4. However, since at higher YO/bp ratio there is a significant amount of YO that is not intercalated, the actual wire length is probably somewhat shorter. Nevertheless, the use of a longer DNA strand requires higher concentrations of YO to fill up the wire and to connect Pacific Blue and Cy3. The YO/bp ratios 0.05 and 0.4 correspond to an average of 2.5 to 20 YO/strand, which is significantly more than that in the shorter strands. Due to the diffusive character of the energy migration, the excitation energy can go both forward and backward. Because of this, a longer wire will lead to a significant increase in the number of FRET steps and a more complex energy transfer profile. Using the same chromophore system as for the 20-mer, Pacific Blue-YO-Cy3, a set of wires is created by simple addition of YO to the Pacific Blue and Cy3-labeled double-stranded DNA. The efficiency of the wire performance is investigated in the same way as for the 20-mer.

Just as in the case with the 20-mer, the emission spectrum for the wire without added YO is dominated by Pacific Blue emission, as shown in Figure 3a. The direct transfer between Pacific Blue and Cy3, present for the 20-mer case, is almost nonmeasurable for the 50-mer wire. At low YO/bp ratios, e.g., 0.05 that corresponds to an average of 2.5 YO/strand, there is quenching of the Pacific Blue emission but only a very small increase in Cy3 emission. Increasing the YO concentration and thus, the YO density in the wire results in further quenching of the Pacific Blue emission and an increase in the Cy3 emission. The slow increase in Cy3 emission of the 50-mer wire compared to the 20-mer is expected. Energy transfer in the 20-mer occurs mainly in a two-step fashion from Pacific blue to Cy3 via YO. In the longer wire, several homotransfer steps are required for end-to-end transfer. Because of this, a higher binding ratio is required in the 50-mer wire, and thus, an increased effect of alternate binding modes and localized energy wells hindering the energy migration in the YO part of the wire is observed. With the increased number of binding sites in the 50-mer, there is also a larger number of chromophore conformations not resulting in energy transfer from Pacific Blue to Cy3. The extracted Cy3 emission for the complete (Pacific Blue-YO-Cy3) wires and the corresponding wires without Pacific Blue are presented in Figure 3b. Since there is almost no direct Pacific Blue to Cy3 energy transfer, the Cy3 intensity for the strand without added YO is virtually zero. In contrast to the 20-mer, the 50-mer shows a slower increase in Cy3 emission without indication of reaching a maximum value. The end-to-end efficiency is estimated to be 29% for the 0.4 YO/bp ratio using eq 2. The fluorescence arising from direct excitation of YO follows the same pattern as the 20-mer with a virtually linear increase with the YO concentration. As the length of the wire increases, so does the number of energy transfer steps needed for the excitation energy to go from one end to the other, and consequently, as discussed for the 20-mer, this results in an increase in the number of potential energy traps and potential escape of the excitation energy before reaching the terminal Cy3. The continuous intensity increase with added YO shows that, for long distances such as this, a large number of highly efficient (i.e., short-range) energy transfer steps are needed for efficient end-to-end transfer.

Time-Resolved Energy Transfer Measurements. The time it takes for the excitation energy to propagate from one end of the wire to the other is determined by the energy transfer rate

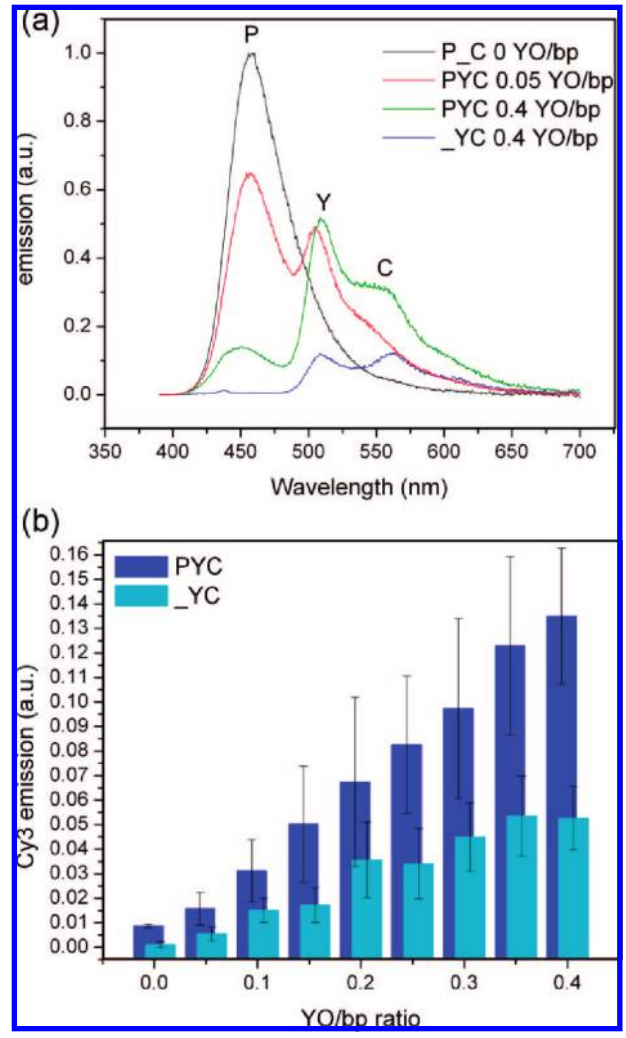

Figure 3. (a) Measured emission spectra of the 50-mer photonic wire for various YO concentrations (excited at 380 nm). Indicated are peaks for Pacific Blue (P), YO (Y), and Cy3 (C), respectively. P_C is the wire with only Pacific Blue and Cy3, _YC is the wire with YO and Cy3 and PYC is the complete wire containing all three chromophores. (b) Extracted Cy3 emission from strands with and without attached Pacific Blue. The data is the average of two separate measurements. The data extraction method used is described in the Materials and Methods section.

and the required number of energy transfer steps. Since our wire construct is based on non-covalent interactions, there will always be an ensemble of different conformations, resulting in a distribution of different transfer times. However, the average transfer time gives an estimate of the wire performance at a certain length, and this is of interest when designing applications. For the short 20-mer wire, energy transfer occurs mainly through a two-step energy transfer from Pacific Blue, through YO, to Cy3. When the strand length is increased, the number of homotransfer steps increases and the diffusive character of energy propagation becomes more pronounced. We have measured the fluorescence decay at a wavelength dominated by Cy3 emission to estimate the time required for the excitation energy to arrive to Cy3 after excitation of Pacific Blue.

The fluorescence decay of the photonic wire is collected at 570 nm using TCSPC, a wavelength with emission primarily from Cy3. Figure 4 shows the fluorescence decay at 570 nm for both the 20-mer and the 50-mer photonic wires.

The fluorescence decays are fitted to a three-exponential expression; two with positive pre-exponential factors and one

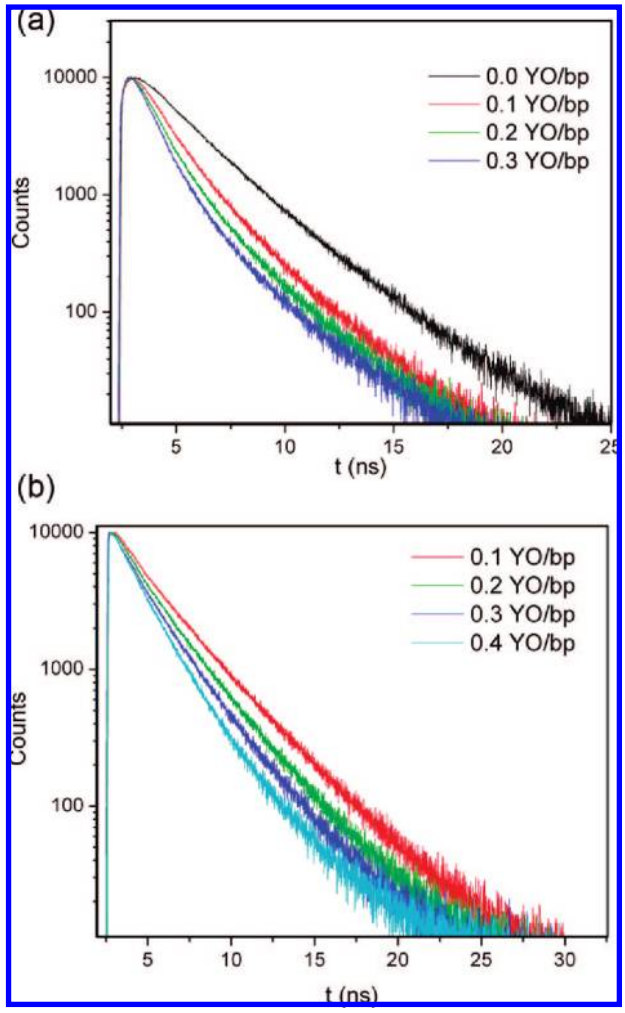

Figure 4. Fluorescence decays measured with excitation at 380 nm and monitoring the emission at 570 nm of the complete 20-mer (a) and 50-mer (b) photonic wires. The fluorescence decays were fitted with three exponentials resulting in reduced χ^2 values <1.1.

with a negative factor. The negative exponential is seen in the time-resolved spectra as a risetime. This behavior with a combination of positive and negative exponential decays can be explained by a consecutive reaction model showing inverted kinetics as shown below and in eq 3.

$$D* \xrightarrow{k_a} Cy3* \xrightarrow{k_b} Cy3$$

$$[Cy3*] = \frac{k_a}{k_b - k_a}(e^{-k_a t} - e^{-k_b t})[D*]_0 \qquad (3)$$

Here, excited Pacific Blue or YO (D*) act as starting points transferring energy to Cy3, which then decays to the ground-state by emission of a photon. The excited Cy3 (Cy3*) is an intermediate between excited Pacific Blue or YO and the final product, which is the wire in its ground state (Cy3). The population of excited Cy3 varies over time as a function of the transfer rate from Pacific Blue and YO to Cy3 and its radiative and nonradiative decay to the ground state. Following the model of consecutive kinetics (eq 3), the observed risetime corresponds to the fluorescence decay of Cy3. The negative decay, which appears as a risetime in the two spectra, was fitted with a single lifetime of 0.3 ns. The value is consistent with the measured

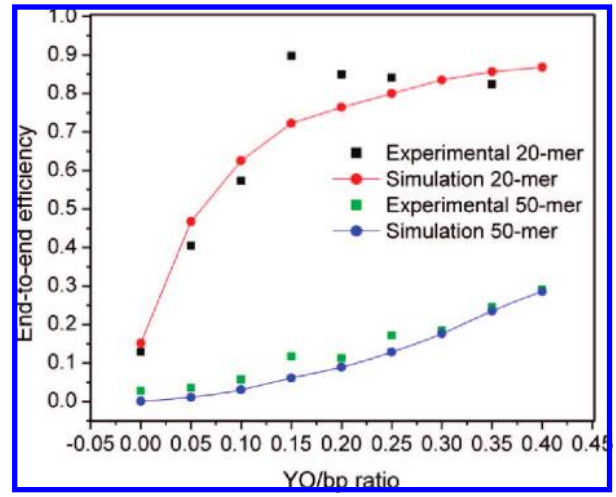

Figure 5. Comparison between simulated and experimental data showing the energy transfer efficiency obtained for different YO concentrations. The red and the blue lines connecting the respective simulation results are only guides to the eye.

lifetime of Cy3 in a Cy3 only wire (data not shown) and with previously made observations of the Cy3 fluorescence lifetime.[26] The two positive components represent the distribution of arrival times from the other two chromophores Pacific Blue and YO. As shown above, there is a detectable direct energy transfer between Pacific Blue and Cy3 and a rapid increase in Cy3 emission when YO is added for the 20-mer. This behavior is also observed in the time-resolved fluorescence measurements. A comparison between the black (0 YO/bp) and red curve (0.1 YO/bp) in Figure 4 shows the substantial increase in transfer rate to Cy3 upon addition of YO. This can be seen from the distinct decrease in the arrival times (i.e., faster decay) when increasing the YO concentration going from an average lifetime of 2.7 ns with no added YO to 1.5 ns for 0.4 added YO/bp. Without any added YO, the arrival time of the excitation energy to Cy3 corresponds to the excited-state lifetime of Pacific Blue in the same strand. However, upon addition of YO, the arrival time does not follow the decrease in lifetime of Pacific Blue (measured at a wavelength were only Pacific Blue emits). This show that when YO is added, the coupling between Pacific Blue and Cy3 is strengthened and that a large fraction of the excitation energy reaching Cy3 comes from multiple energy transfers through YO.

Figure 4b shows the corresponding fluorescence decays of the 50-mer. Here, the same trend as for the 20-mer is observed and the decays can be fitted with two positive exponentials and one negative. However, the negative component, corresponding to the excited-state lifetime of Cy3, is less pronounced than that for the 20-mer wire, reflecting the weaker Cy3 emission for the 50-mer wire. The two positive fluorescence decays are also fitted with longer lifetimes than that for the 20-mer. As expected, the average arrival time obtained from the two positive exponentials used to fit of the fluorescence decay at 570 nm decrease with increasing YO concentration. At 0.1 YO/bp, the average lifetime was 2.7 ns, showing a monotonous decrease to 1.9 ns for 0.4 YO/bp. The arrival times are longer for the 50-mer than for the 20-mer, since the number of energy transfer steps needed to reach from Pacific Blue to Cy3 increase with increasing wire length. However, because the signal from Cy3 is relatively weak and there is substantial overlap between YO and Cy3 emission, it is not possible to draw quantitative conclusions from the determined lifetimes.

Simulation of Excitation Energy Transfer. In addition to the experimental data, we have used a Markov chain model that simulates the end-to-end excitation energy transfer in this kind of system. Förster distances for the chromophore combination Pacific Blue-YO (52 Å), Pacific Blue-Cy3 (51 Å), and YO-Cy3 (59 Å) was determined experimentally. The YO−YO Förster distance (37 Å) has been determined elsewhere.[28] The different Förster distances were used as experimental inputs in the simulation. A semiempirical model gives the opportunity to predict the behavior of a wire with an arbitrary length, which is beneficial when designing a more complex system. The model accounts for both the direct energy transfer from Pacific Blue to Cy3 and, more importantly, the energy transfer relayed through intercalated YO molecules. Excitation energy at a YO position can, apart from being emitted, go to any other YO chromophore or to Cy3, where it exits the system. The fraction of the initial excitation energy that arrives at the Cy3 position is used as a measure of the wire efficiency. Since the intercalators insert randomly into the DNA strands, different wires in a sample will not have the same distribution of chromophores, both with regard to the positioning of the intercalators and with regard to the number of intercalators. This is accounted for in the simulation by using a Poisson distribution to generate the actual number of intercalators around an average binding ratio. Unique chromophore positions are also given for every round of the simulation to account for the different possible wire conformations. By averaging a large number of simulations, an estimate of the end-to-end transfer efficiency is obtained (Figure 5).

To evaluate the performance of the theoretical model, a comparison with experimental data was made. The energy transfer efficiency was calculated from the extracted Cy3 emission with the contribution from direct excitation of YO subtracted as described in the Methods section. This gives an experimental estimation of the end to end transfer efficiency. As can be seen in the comparison between measured and simulated data, the experimental data fit very well with the theoretical model for the two wire lengths. The transfer from Pacific Blue to Cy3 is very efficient for the shorter strand, showing 85% efficiency in the simulation at 0.4 YO/bp. For the 50-mer wire, about 29% of the excitation energy reaches Cy3 at this YO/bp ratio. The simulation of the Pacific Blue-YO-Cy3 wire only determines the transfer going through intercalated YO. However, as stated above, at high binding densities, there are other binding modes having a significant impact on the energy transfer behavior. This is most evident for the longer wire where high YO binding density is required for end-to-end energy transfer to occur. Our experimental data showed lower transfer efficiency for the long wire than would be expected for complete intercalation. This can be explained by the existence of trap states adding additional ways for YO to nonradiatively decay. To account for this, a loss term was included in the simulation increasing the decay rate of YO with increasing intercalator density. A detailed description of the loss term is given in the Supporting Information. Without this loss term, simulation of energy transfer results in wires with 90% efficiency for the 20-mer and 37% efficiency for the 50-mer at a YO/bp ratio of 0.4. A direct comparison of wire efficiencies obtained here with those from previously performed studies on similar systems is not straightforward. DNA-based photonic wires display a high heterogeneity[16,20] and energy transfer estimates depend both on the nature of the studied system and on the experimental method. Using covalently attached chromophores arranged for heterotransfer, Sauer and co-workers report a 40 base pair wire working at 68% efficiency with a 45% yield.[21] In photonic wires, applying covalent homotransfer approaches, overall transfer efficiencies of approximately 20% have been obtained for wires of 30[24] and 40[23] base pair lengths. From this, it can be concluded that our wire using intercalating chromophores gives efficiencies as high as covalent counterparts and shows energy transfer over even longer wire lengths.

Conclusions

We have designed a simple and highly flexible method to create a nanoscale optical wire using double-stranded DNA as a scaffold for a series of interconnected chromophores. The use of an intercalator, YO, rather than covalently attached dyes as a relay, increases the simplicity of the wire assembly and gives greater flexibility in wire length. Here, we have demonstrated a self-assembled wire capable of end-to-end energy transfer over distances of ∼20 nm. The results show that the transfer efficiency of our self-assembled system is comparable to wires using covalently attached dyes which, in contrast, require a more complex molecular design and assembly processes, as well as more advanced synthetic work. Moreover, a theoretical model that successfully simulates the energy transfer of our wires was presented. The model can be a valuable tool for predicting wire behavior in future wire design. The wire efficiency is greatly dependent on the number and length of the involved energy transfer steps. Moreover, the presence of competing binding modes for YO and energy traps also influences the transfer efficiency for the longer wires. There are several development opportunities that could address this issue; an intercalator with a larger Förster critical distance would decrease the number of chromophores and, therefore, the number of energy transfer steps required for end-to-end transfer. An intercalator that is completely intercalated up to saturation of the DNA strand would allow for closer packing of the chromophores with more efficient energy transfer and no competing binding modes that may function as an energy escape route. Apart from improving wire performance, there are a few possible ways to develop the DNA wire concept. Using branched DNA structures and other more complex DNA assemblies[31] as scaffolds for the photonic wires may allow for more complicated self-assembled photonic circuitry to be constructed. The DNA photonic wires could also be useful when incorporated into nanofabricated surfaces.[32] Interesting applications could be envisioned by using the photonic wires to direct excitation energy and thereby control photochemical reactions mimicking the function of natural light harvesting antenna systems.

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Supporting Information Available: The simulation used to estimate the end-to-end transfer efficiencies in the different wire constructs is described in detail. This information is available free of charge via the Internet at http://pubs.acs.org.

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