DNA Based Artificial Light-Harvesting Antenna

by

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ABSTRACT

Scientists around the world have been striving to develop artificial light-harvesting antenna model systems for energy and other light-driven biochemical applications. Among the various approaches to achieve this goal, one of the most promising is the assembly of structurally well-defined artificial light-harvesting antennas based on the principles of structural DNA nanotechnology. DNA has recently emerged as an extremely efficient material to organize molecules such as fluorophores and proteins on the nanoscale. It is desirable to develop a hybrid smart material by combining artificial antenna systems based on DNA with natural reaction center components, so that the material can be engineered to convert light energy to chemical energy via formation of a charge-separated state.

Presented here are a series of studies toward this goal. First, self-assembled seven-helix DNA bundles (7HB) with cyclic arrays of three distinct chromophores were developed. The spectral properties and energy transfer mechanisms in the artificial light-harvesting antenna were studied extensively using steady-state and time-resolved methods. Next, engineered cysteine residues in the reaction center of the purple photosynthetic bacterium *Rhodobacter sphaeroides* were each covalently conjugated to fluorophores in order to explore the spectral requirements for energy transfer between an artificial light harvesting system and the reaction center. Finally, a structurally well-defined and spectrally tunable artificial light-harvesting system was constructed, where multiple organic dyes were conjugated to 3-arm DNA nanostructure. A reaction center protein isolated from the purple photosynthetic bacterium *Rhodobacter sphaeroides* was linked to one end of the 3-arm junction to serve as the final acceptor, which converts the
photonic energy absorbed by the chromophores into chemical energy by charge separation. This type of model system is required to understand how parameters such as geometry, spectral characteristics of the dyes, and conformational flexibility affect energy transfer, and can be used to inform the development of more complex model light-harvesting systems.
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Chapter 1

Artificial Light-Harvesting Antenna and DNA Nanotechnology

1.1. Artificial Light-Harvesting Antenna

1.1.1. Introduction: One of the very important steps in natural photosynthesis is the absorption of sunlight by light-harvesting complexes, which is shuttled around the network of light-harvesting complexes until trapped at reaction center complex, where the light energy is converted to chemical energy via charge separation. The resulting potential is used to pump protons across the membrane, which is eventually utilized in adenosine triphosphate (ATP) synthesis (Figure 1.1). The most abundant and sustainable source of energy to mankind is sunlight, hence making a device to utilize the enormous energy coming from the sun for the human race is a great challenge at this time. One obvious approach is to mimic the natural light-harvesting antenna systems and their essential features to develop smart bio-inspired materials for energy applications. The natural light-harvesting antenna contains huge numbers of bacteriochlorophyll molecules per reaction center complex. These sophisticated supramolecular structures are very efficient in low photon density and transfer energy unidirectionally toward the reaction center. It is important to know that the high efficiency of energy flow in natural antenna systems is due to the well-defined organization of a multitude of chromophores with distinct optical and redox properties. These processes are not only efficient, they are often ultrafast in order to cover large distances in a very short time span. Therefore an artificial light-harvesting antenna should have well-organized chromophores with well-defined inter-chromophoric distances and with known ratios of multitude of donors and acceptors, to have high energy transfer efficiency.
Figure 1.1. Schematic of electron flow in purple photosynthetic bacteria. The horizontal band depicting the lipid bilayer containing various protein components. The proton gradient created by reaction center is utilized in ATP synthesis. Figure reprinted from Reference 8. Copyright Sinauer Associates, Inc.

1.1.2. Examples of Artificial Antenna Systems: Supramolecular systems created by synthetic organic chemistry come with high geometric and design precision in terms of arranging dye molecules. There are an enormous amount of published reports available on light-harvesting antennas created by using organic synthesis. Among them one important example is a dendrimer that incorporates discrete dye molecules in the form of layers.\textsuperscript{12,17-22} Dendrimers are branched synthetic macromolecules having many chain ends originated from a central core. Thus, by placing a final acceptor in the center surrounded by arrays of donor molecules, the dendrimer antenna has the capability of absorbing light and funneling that energy to a central point. Figure 1.2 shows the structure of a multi-chromophoric poly(aryl ether) dendrimer containing Coumarin-2 (\(\lambda_{\text{abs,max}} = 350\ \text{nm}, \lambda_{\text{em,max}} = 440\ \text{nm}\)) and Fluorol-7GA (\(\lambda_{\text{abs,max}} = 415\ \text{nm}, \lambda_{\text{em,max}} = 520\ \text{nm}\)) as donors at the third and second branch point, respectively, and a perylene core (\(\lambda_{\text{abs,max}} = 555\ \text{nm}, \lambda_{\text{em,max}} = 610\ \text{nm}\)) as the
acceptor. The fluorophores were chosen in such a way that the absorbed energy by Coumarin-2 would be transferred to the central perylene core via intermediate Fluorol-7GA through step-wise energy transfer. Due to the high spectral overlap between the emission of Coumarin-2 and the absorbance of Fluorol-7GA and a close inter-chromophore distance, a 99% energy transfer efficiency is observed. Similarly, the energy transfer efficiency from Fluorol-7GA to central perylene is 96%, again due to high spectral overlap and close distance. There is a smaller direct energy transfer (79%) from the initial donor to the central core. Thus, dendrimers with multitude of chromophores surrounding a single core can be an important building block for artificial light-harvesting system.

**Figure 1.2.** Molecular structure of fluorophore substituted dendrimer illustrating the FRET efficiencies between Fluorophores. Reproduced from Reference 21. Copyright 2002 The Royal Society of Chemistry.

Porphyrrins have been used in various light-harvesting materials due to their spectroscopic properties. Including porphyrin-based dendrimers, there are enormous amounts of reports on porphyrin-based supramolecular structures for light-harvesting and
charge separation.\textsuperscript{11,23-34} Figure 1.3 shows structures of different cyclic porphyrin arrays as models of light-harvesting antenna complexes. Many of those arrays are chemically robust and have controlled inter-porphyrin orientations and distances, making them extremely important for light-harvesting studies. The major drawback with the supramolecular synthesis approach is the difficulty in synthesizing monodispersed structures with extremely large numbers of unique chromophores. Another excellent approach to make artificial antenna system is constructing fluorophore incorporated nano-disks and -rods by

![Figure 1.3. Structure of different porphyrin arrays. Reproduced from Reference 23. Copyright 2009 American Chemical Society.](image)

**Figure 1.3.** Structure of different porphyrin arrays. Reproduced from Reference 23. Copyright 2009 American Chemical Society.

![Figure 1.4. Self-assembly of TMVP monomers labeled with donor and acceptor dye forms rod shaped light-harvesting structures. Reproduced from Reference 37. Copyright 2007 American Chemical Society.](image)

**Figure 1.4.** Self-assembly of TMVP monomers labeled with donor and acceptor dye forms rod shaped light-harvesting structures. Reproduced from Reference 37. Copyright 2007 American Chemical Society.
self-assembling proteins. Tobacco mosaic virus coat protein (TMVP) is an ideal platform for such constructs.\textsuperscript{33,35-37} The disk- or rod-shaped structures were constructed by conjugating chromophores to cysteine residues introduced on TMVP monomers followed by self-assembly of the monomers under appropriate buffer conditions (Figure 1.4). Additionally, light-harvesting nanotubes consisting of double-walled nanotubular dye aggregates have been developed recently.\textsuperscript{38} However, it still remains a challenge to organize chromophores with high precision and predictability, which are the key factors that determine the energy transfer efficiency in the designed artificial light-harvesting antenna system.

1.2. DNA Nanotechnology and Its Application Towards Artificial Antenna

1.2.1. DNA Nanotechnology: Deoxyribonucleic acid, or DNA, the ‘blue-print’ of life, is one of the most important biopolymers, which is an excellent material for creating nanostructures. In 1982, Nadrian Seeman first proposed that it is possible to construct immobile junctions using DNA with asymmetric sequences\textsuperscript{39} through Watson-Crick base pairing\textsuperscript{40} between complementary DNA strands, giving birth to the field of DNA nanotechnology. Figure 1.5 shows the self-assembly of double helical DNA molecules through sticky end (unhybridized single strand part, bases shown in purple color) cohesion. If the single stranded fragments are complementary to each other, then mixing the two double helical DNA structures will lead to a single double helical structure. Over three decades after Seeman’s proposed idea, enormous amounts of different DNA-based nanostructures are emerging, ranging from bundles, nanotubes, and 2D lattice arrays to 3D nanostructures (Figure 1.6 A and B).\textsuperscript{41-50}
Figure 1.5. Self-assembly of two double helical DNA molecules through sticky end cohesion.
Figure 1.6. (A) Schematic showing the self-assembly of nanostructures based on DNA base pairing. (B) Examples of DNA bundles and nanowire (left), 2D arrays (middle), and 3D nanostructures. Self-assembly of a long scaffold strand and hundreds of staple strands are leading to various 2D (C) and 3D (D) DNA nanostructures. Reproduced from Reference 50. Copyright 2012 American Chemical Society.

A breakthrough was made in 2006, when Paul Rothemund came up with the idea of folding long single-stranded M13mp18 genomic DNA (7249 bases) into arbitrary 2D shapes by a collection of short oligonucleotides, which are called ‘staple strands’ (Figure 1.6 C).51 These discrete structures with preprogrammed shapes and sizes are called ‘DNA origami’. Within a few years of that innovation, the origami approach had been extended to create 3D nanostructures.52-55

DNA nanotechnology not only has been used to create outstanding, beautiful structures; these structures were also used extensively to organize other entities, such as nanoparticles, quantum dots, carbon nanotubes, proteins, viral capsids and many other functional molecules.49,50,56-67 Chemical modification of oligonucleotides to conjugate chromophores is routinely performed, and due to the spatial addressability of programmable DNA nanostructures, it is possible to organize a multitude of chromophores and other light-harvesting materials to create artificial light-harvesting devices.

1.2.2. DNA Based Photonic Devices: The simplest photonic devices are photonic wires, which are created by arranging fluorophores linearly so that light energy is transported from one end to the other. The first report of a molecular photonic wire was based on porphyrin arrays.68 After that, DNA has been used widely to arrange fluorophores in a programmable fashion to create photonic devices.69-72 Fluorophore selection in the
creation of photonic wires has to be done in such a way that there is an energy downhill from one end to the other, which means that the fluorophore at the primary absorption end has to be in the high energy end. There are two ways to arrange fluorophores in a photonic wire linearly: one is conjugating the dyes to the DNA covalently, and the other is by intercalation between bases.

**Figure 1.7.** (A) Schematic of DNA-based photonic wire consisting of Pacific Blue, YO-PRO and Cy3. (B) Energy level diagram showing excitation of the donor Pacific blue and subsequent energy transfer to Cy3 acceptor through migration of energy in YO section of the wire. (C) Simulated and experimental data showing end-to-end energy transfer efficiency for 20-mer and 50-mer DNA wires with varying YO concentration. Adapted from Reference 72. Copyright 2008 American Chemical Society.
Figure 1.8. Schematic of quantum dot (QD) with multi-dye-labeled double stranded DNA showing energy transfer pathways from QD donor to the final acceptor via intermediate fluorophores. Adapted from Reference 73. Copyright 2010 American Chemical Society.

For the former case, energy transfer will happen through hetero-FRET (Förster Resonance Energy Transfer), and for the later one the energy transfer will happen via homo-FRET. Figure 1.7A shows the schematic of a photonic wire based on both homo- and hetero-FRET. In this system, pacific blue is acting as an initial donor, and the excited energy is transferred to Cy3 via the intermediate intercalator YO-PRO. Both 20-mer and 50-mer double stranded DNA-based photonic wires showed significant energy transfer and the FRET efficiency varied with the intercalator/DNA ratio (Figure 1.7B).

Other than fluorophores, quantum dots also have been studied as primary donors (Figure 1.8).\textsuperscript{73} In this work, double stranded DNA-peptide constructs with fluorophores self-assembled on QDs via metal-Histidine affinity coordination. Each increasingly red shifted dye was chosen to have significant spectral overlap between the emission of donor and the absorption of acceptor.

In the year 2011, Tinnefeld et al. demonstrated the control of energy transfer direction by studying single-molecule four color FRET using DNA origami as a template (Figure 1.9).\textsuperscript{74} A blue fluorophore (ATTO488) was used as the input dye, and a red fluorophore (ATTO647N) and an infra-red (IR) fluorophore (Alexa 750) were used as the alternative output dyes. A green fluorophore (ATTO565) was used as a jumper dye, which can be placed between blue and red, or/and between blue and IR. The alternative energy-transfer paths were visualized by using four-color single molecule FRET approach. No energy transfer from the input to the output dyes was observed in the absence of the jumper
dye, but upon inserting the jumper dye between the input and the output dyes, it clearly showed direction-specific energy-transfer based on the location of the jumper dye. It is important to mention that, in the case of two inserted jumper dyes, signals from both the output dyes were observed.

**Figure 1.9.** Schematic of the arrangement of fluorophores on the DNA origami, in which the “jumper” dye (green ball) dictate the direction of energy flow. The right panel showing FRET-related ratios from blue to red and/or IR in presence and absence of the green dye. Adapted from Reference 74. Copyright 2011 American Chemical Society.

### 1.2.3. DNA Based Light-Harvesting Complexes:

Inspired by nature there have been several examples of creating light-harvesting complexes using DNA nanostructures. Our own work, described in Chapter 2, is one of the earliest reports on complex DNA nanostructures with a multitude of photonic elements toward the creation of artificial light-harvesting antenna. Following this, there are some more recent efforts towards that goal. For example, figure 1.10A shows the schematic of a DNA three-way junction with a $\pi$-stacked multichromophoric array (phenanthrene, yellow). The green disk represents the acceptor dye, which is either pyrene or perylenediimide or Cy3. The phenanthrene excitation at 320 nm is followed by energy transfer to pyrene,
Fluorescence emission change was studied by varying the molar ratio of the pre-annealed red and blue strands to the green single strand. A gradual increase of Cy3 fluorescence is observed up to equimolar concentrations.

Another approach in creating light-harvesting system is to make a hybrid structure consist of DNA scaffolds and lipid bilayer (Figure 1.10B). Duplex DNA with intercalated YO is placed on lipid bilayer by using porphyrin as an anchor. The intercalating dye YO helps to migrate the excitation energy along the duplex and finally transfer to the porphyrin acceptor. This approach has potential to be used for future developing of surface-associated artificial reaction centers or light-harvesting systems.

**Figure 1.10.** DNA based light-harvesting antenna systems. (A) Schematic of light harvesting three-way junction (3WJ). Adapted from Reference 79. Copyright 2014 The Royal Society of Chemistry. (B) Schematic showing a duplex DNA with seven intercalated YO dye is bound to lipid bilayer via porphyrin anchoring. Adapted from Reference 31. Copyright 2013 American Chemical Society.

### 1.3. Brief Introduction of Projects Covered in This Dissertation

It is the aim of this dissertation work to explore seek the possibilities of combining DNA nanotechnology and photonic elements to create artificial light-harvesting antenna systems. The following section summarizes the projects executed in this context.
1.3.1. DNA Directed Artificial Light-Harvesting Antenna (Chapter 2):
Designing and constructing multi-chromophoric, artificial light-harvesting antennas with controlled inter-chromophore distances, orientations and defined donor-acceptor ratios to facilitate efficient unidirectional energy transfer is extremely challenging. Here, we demonstrate the assembly of a series of structurally well-defined artificial light harvesting triads based on the principles of structural DNA nanotechnology. DNA nanotechnology offers addressable scaffolds for the organization of various functional molecules with nanometer scale spatial resolution. The triads are organized by a self-assembled seven helix DNA bundle (7HB) into cyclic arrays of three distinct chromophores, reminiscent of natural photosynthetic systems. The scaffold accommodates a primary donor array (Py), secondary donor array (Cy3) and an acceptor (AF) with defined inter-chromophore distances and orientations. Steady-state fluorescence analyses of the triads revealed an efficient, step-wise funneling of the excitation energy from the primary donor array to the acceptor core through the intermediate donor. The efficiency of excitation energy transfer and the light harvesting ability (antenna effect) of the triads was greatly affected by the relative ratio of the primary to the intermediate donors, as well as the inter-chromophore distance. Time resolved fluorescence analyses by time correlated single photon counting (TCSPC) and a streak camera further confirmed the cascading energy transfer processes on the picosecond time scale. Our results clearly show that DNA nanoscaffolds are promising templates for the design of artificial photonic antennas with structural characteristics that are ideal for the efficient harvesting and transport of energy.

1.3.2. Reengineering the Optical Absorption Cross-section of Photosynthetic Reaction Centers (Chapter 3): Engineered cysteine residues near the primary electron
donor (P) of the reaction center from the purple photosynthetic bacterium *Rhodobacter sphaeroides* were covalently conjugated to each of several dye molecules in order to explore the geometric design and spectral requirements for energy transfer between an artificial antenna system and the reaction center. An average of 2.5 fluorescent dye molecules were attached at specific locations near P. The enhanced absorbance cross-section afforded by conjugation of Alexa Fluor 660 dyes resulted in a 2.2-fold increase in the formation of the reaction center charge-separated state upon intensity-limited excitation at 650 nm. The effective increase in absorbance cross-section resulting from the conjugation of two other dyes, Alexa Fluor 647 and Alexa Fluor 750, was also investigated. The key parameters that dictate the efficiency of dye-to-reaction center energy transfer and subsequent charge separation were examined using both steady-state and time-resolved fluorescence spectroscopy as well as transient absorbance spectroscopy techniques. An understanding of these parameters is an important first step towards developing more complex model light-harvesting systems integrated with reaction centers.

### 1.3.3. A DNA-Directed Light-Harvesting/Reaction Center System (Chapter 4):

A structurally well-defined and spectrally tunable artificial light-harvesting system is constructed, where multiple organic dyes are conjugated to a 3-arm DNA nanostructure, and the reaction center isolated from the purple photosynthetic bacterium *Rhodobacter sphaeroides* 2.4.1 (PDB 2J8C) is linked to one end of the DNA 3-arm junction as the final acceptor, which converts the absorbed photonic energy by the chromophores into a chemical form via charge separation. The number of DNA 3-arm junctions per reaction center was tuned from 1 to 3. This DNA-templated multi-chromophore system is demonstrated to serve as a modular light-harvesting antenna that is capable to optimize the
spectral properties, energy transfer efficiency and photo-stability of the system and broadens the spectral regime of light-induced electron transfer of the reaction center. This device has future applications in electrical energy production and light driven bio-catalysis.

1.4. References


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Chapter 2

DNA-Directed Artificial Light-Harvesting Antenna


2.1. Abstract

Designing and constructing multi-chromophoric, artificial light harvesting antennas with controlled inter-chromophore distances, orientations and defined donor-acceptor ratios to facilitate efficient unidirectional energy transfer is extremely challenging. Here, we demonstrate the assembly of a series of structurally well-defined artificial light harvesting triads based on the principles of structural DNA nanotechnology. DNA nanotechnology offers addressable scaffolds for the organization of various functional molecules with nanometer scale spatial resolution. The triads are organized by a self-assembled seven helix DNA bundle (7HB) into cyclic arrays of three distinct chromophores, reminiscent of natural photosynthetic systems. The scaffold accommodates a primary donor array (Py), secondary donor array (Cy3) and an acceptor (AF) with defined inter-chromophore distances and orientation. Steady state fluorescence analyses of the triads revealed an efficient, step-wise funneling of the excitation energy from the primary donor array to the acceptor core through the intermediate donor. The efficiency of excitation energy transfer and the light harvesting ability (antenna effect) of the triads was greatly affected by the relative ratio of the primary to the intermediate donors, as well as on the inter-chromophore distance. Time resolved fluorescence analyses by time correlated single photon counting (TCSPC) and a streak camera further confirmed the cascading
energy transfer processes on the picosecond time scale. Our results clearly show that DNA nanoscaffolds are promising templates for the design of artificial photonic antennas with structural characteristics that are ideal for the efficient harvesting and transport of energy.

2.2. Introduction

Photosynthesis is one of the most fascinating photochemical events in nature. It is initiated by the absorption of visible light by antenna units comprised of a large number of pigment molecules, followed by funneling of the excitation energy within the antenna assembly to a reaction center where optical energy is converted to chemical energy.\(^1\)-\(^3\) The very high efficiency of natural photosynthesis is a consequence of the well-defined organization of a multitude of chromophores with distinct optical and redox properties that facilitate the efficient capture of visible light and the subsequent transfer of the excitation energy. Artificial light harvesting systems with multiple chromophores have been found to display unidirectional energy transfer,\(^4\) and may have potential applications with the conversion of light into chemical potentials.\(^5\) Dendrimers have been explored as covalent scaffolds for constructing arrays of chromophores that exhibit high energy transfer rates and directionality, however it is very difficult to synthesize monodispersed dendrimers containing a large number of unique chromophores.\(^6\) Self-assembling proteins, such as Tobacco Mosaic Virus Coat protein, have recently been demonstrated as excellent alternative scaffolds for the construction of multi-chromophore artificial light harvesting antennas, offering the ability to organize several thousands of chromophores.\(^7\)-\(^9\) However, it still remains a challenge to organize multiple chromophores into arrays with well-defined inter-chromophore distances, control their relative orientations and the exact ratio of
donors to acceptors, which are the key factors that determine the efficiency of FRET (Förster Resonance Energy Transfer).

Structural DNA nanotechnology,\textsuperscript{10-12} including the DNA ‘origami’ approach,\textsuperscript{13} has developed to the point that fully addressable nano-architectures of various geometries can be easily designed and constructed. DNA nanostructures have been used as scaffolds for the directed self-assembly of many nanomaterials including nanoparticles,\textsuperscript{14,15} quantum dots,\textsuperscript{16,17} carbon nanotubes,\textsuperscript{18} proteins\textsuperscript{19-27} and viral capsids,\textsuperscript{28} and other functional molecules.\textsuperscript{29} Modified phosphoramidite building blocks are commercially available and chemical modification of oligonucleotides is routinely performed with a DNA synthesizer following standard procedures. Therefore, it is relatively easy to generate chromophore labeled oligonucleotides for subsequent incorporation into DNA nanoscaffolds, e.g. through sequence specific hybridization. The DNA scaffolds are ideal platforms to organize arrays of multiple chromophores because of their ability to tune the distance and relative orientation between the chromophores with remarkable precision. Although there have been reports of the DNA templated organization of fluorophores into unidirectional photonic wires, it is still a challenge to engineer a large number of fluorophores into 3D geometries.\textsuperscript{30}

Here we report the DNA templated design and construction of a series of discrete and structurally well-defined light harvesting systems that each consists of three different types of chromophores, organized in a manner similar to the natural light harvesting antenna. The unidirectional, step-wise energy transfer from an array of primary donors to a single acceptor through an intermediate array of secondary donors is clearly demonstrated
by steady state and time resolved spectral analysis. A systematic study of the effect of donator-acceptor ratios on the efficiency of FRET is also presented.

2.3. Materials and Methods

2.3.1. Dye Modified DNA Synthesis, and DNA Structure Preparation and Purification: Unmodified oligonucleotides and AF modified oligonucleotides were purchased from Integrated DNA Technologies, Inc. (www.idtDNA.com) and purified by 10% denaturing PAGE. The designed structures were prepared by mixing stoichiometric quantities of 19 DNA strands in TAE/Mg\(^{2+}\) buffer (40 mM Tris base, 20mM Acetic acid, 2 mM EDTA·Na\(_2\)·12H\(_2\)O, 63.4 mM (CH\(_3\)COO)\(_2\)Mg·4H\(_2\)O). High Mg\(^{2+}\) concentrations were used to overcome the repulsion between the negatively charged DNA backbones. After mixing, the final concentration of each single strand is 1\(\mu\)M. The solution was subsequently annealed from 90 °C to room temperature over 12 hr. After annealing the structures were purified by 5% non-denaturing PAGE. Chromophore modified (Py and Cy3) oligonucleotides were synthesized on a DNA synthesizer (ABI 394 DNA/RNA Synthesizer, Applied Biosystems) via standard phosphoramidite protocols by using CPGs (1 \(\mu\)mol) with a coupling time of 10 min for each chromophore modified phosphoramidite. The chemicals for DNA synthesis were purchased from Glen Research and were used as received. After preparation, the trityl-off oligonucleotide was cleaved from the resin by treatment with concentrated NH\(_4\)OH (28%) for 24 h at room temperature. Oligonucleotides were purified on an Agilent Technologies 1200 series reverse phase HPLC system using a ZORBAX SB-C18 column (Solvent A: 50mM ammonium acetate, pH 7; Solvent B: acetonitrile, Flow rate: 3 mL/min). After purification the oligonucleotides were lyophilised and quantified by their absorbance at 260 nm. MALDI analyses were carried out on.
Applied Biosystem Voyager System 4320 using 3-hydroxypicolinic acid as the matrix with an accelerating voltage of 25000V.

2.3.2. Absorption and Fluorescence Measurements: Absorption spectra were measured using a quartz cell of 1 cm path length on a Jasco V-670 spectrophotometer. The steady state fluorescence spectra were measured using a Nanolog fluorometer, (Horiba Jobin Yvon, L-format, equipped with a CW 450W Xenon light source, thermoelectrically cooled R928 PMT, and fully automated excitation and emission polarizers for anisotropy measurement) with a quartz cell of 1 cm path length, and all the spectra were corrected for the wavelength dependence of the detection system response.

2.3.3. Fluorescence Life-time Measurements: All the fluorescence life-time decay measurements were analyzed by two instruments. First, by a time-correlated single-photon-counting (TCSPC) method using Titanium Sapphire kilohertz laser system (Millennia/Tsunami, Spectra Physics) with a 130 fs pulse duration operated at 80 MHz. The laser output was tuned to 740 nm and sent through a frequency doubler and pulse selector (Spectra Physics, Model 3980) to obtain 370 nm excitations at 4 MHz. Fluorescence emission was collected at a right angle to the excitation beam and detected using a double-grating monochromator (Jobin-Yvon, Gemini-180) and a microchannel plate photomultiplier tube (Hamamatsu R3809U-50). Data acquisition was performed using a single photon counting card (Becker-Hickl, SPC-830). The instrument response function (IRF) had a full width at half-maximum (FWHM) of 35-45 ps, as verified by scattering from samples.

Fluorescence was also analyzed by an ultrafast laser equipped with a streak camera. The laser beam was generated by a mode-locked Ti:Sapphire laser (Mira 900,
Coherent) pumped by a frequency-doubled Nd:YVO$_4$ laser (44% from an 18 W Verdi, Coherent). The 130 fs light pulses (at 800 nm with a repetition rate of 250 KHz) were generated by a regeneratively amplified Ti:S laser system (RegA 9000, Coherent Laser). The pulses were sent to an optical parametric amplifier (OPA) to generate the excitation light at the desired wavelength. Fluorescence was collected at a right angle to the excitation beam and focused on the entrance slit of a Chromex 250IS spectrograph which was coupled to a Hamamatsu C5680 streak camera with a M5675 synchroscan sweep unit. The streak images were recorded on a Hamamatsu C4742 CCD camera. Measurements were performed on a 2 ns timescale. The FWHM of the overall time response of this system was ~20 ps at the 2 ns timescale. The global analysis was performed using the home-written software package ASUFIT.

**2.3.4. Calculation of FRET Efficiency, Quantum Yield (Q.Y.) and the Antenna Effect:** FRET efficiencies (E) were calculated according to the following equation:

$$E = 1 - \frac{I_{DA}}{A_{DA}} \frac{A_{D}}{I_{D}}$$

Where $I_{DA}$ and $I_{D}$ are the integrated area of donor (Py) fluorescence emission between 390 nm and 500 nm with and without acceptors. The excitation wavelength was 380 nm. $A_{DA}$ and $A_{D}$ are the absorbance of donor (Py) at 380 nm with and without acceptors.

The quantum yield (Q.Y.) of Py and Cy3 were calculated using the following equation:

$$\Phi = \Phi_r \frac{I_A \eta_r^2}{I_r A \eta_r^2}$$
Where Φ and Φ_\text{R} are the Q.Y. of the sample and the reference. Here I and I_\text{R} are the intensities of sample and reference, and A and A_\text{R} are the absorbances of sample and reference at the excitation wavelength. η and η_\text{R} are the refractive indexes of solvents for sample and reference, respectively.

The overall antenna effect (AE1) was calculated using the following equation:

$$ AE1 = \frac{I_{\text{AF,380nm}}}{I_{\text{AF,620nm}}} $$

Where $I_{\text{AF,380nm}}$ and $I_{\text{AF,620nm}}$ are the fluorescence intensities of AF upon excitation of the donor (Py) at 380 nm and the direct excitation of AF at 620 nm for the same sample. The corresponding antenna effect of the second energy transfer step (AE2) with Cy3 excitation at 500 nm was calculated according to the following equation:

$$ AE2 = \frac{I_{\text{AF,500nm}}}{I_{\text{AF,620nm}}} $$

Where $I_{\text{AF,500nm}}$ and $I_{\text{AF,620nm}}$ are the fluorescence intensities of AF upon excitation of the donor (Cy3) at 500 nm and the direct excitation of AF at 620 nm for the same sample.

2.4. Results and Discussion

The chromophores used in this study include ethynylpyrene (Py)\textsuperscript{31} as the primary donor ($\lambda_{\text{max,abs}} = 400$ nm, $\lambda_{\text{max,em}} = 438$ nm), a cyanine derived dye (Cy3) as the intermediate donor ($\lambda_{\text{max,abs}} = 550$ nm, $\lambda_{\text{max,em}} = 566$ nm) and Alexa Fluor 647 (AF)\textsuperscript{32} as the acceptor ($\lambda_{\text{max,abs}} = 650$ nm, $\lambda_{\text{max,em}} = 668$ nm) (Figure 2.1A). The absorption and emission profiles of the chromophores show well-separated absorption and emission characteristics (Figure 2.1B). The spectra also reveal significant overlap between the emission of Py/absorption of Cy3, and the emission of Cy3/absorption of AF, with minimal spectral overlap between Py and AF. The spectral features of the system enable
the selective excitation of distinct chromophores within the self-assembled structure, which is crucial for the examination of the cascade of energy transfer. A precise arrangement of the chromophores results in the efficient, unidirectional step-wise FRET from Py to Cy3 and from Cy3 to AF, with a small amount of direct FRET between Py and AF, especially when they are placed far apart. Furthermore, the three chromophores collectively absorb light throughout the entire visible spectrum (from ~350 nm to ~700 nm) resembling the natural light harvesting antenna.

Figure 2.1. (A) Chemical structure of the chromophores used in this study. (B) Normalized absorption (solid line) and emission (dotted line) spectra for Py (blue), Cy3 (green), and AF (pink) modified DNAs showing the optical spectral overlap. These spectra were obtained using the dye labeled ssDNA hybridized to their complementary ssDNA strands. (See Figure S2.9 for the spectral data of the dye labeled ssDNA).

We used a seven-helix bundle (7HB) motif designed by Seeman and co-workers as the underlying DNA nanoscaffold.33 The schematics shown in Figures 2.2 and 2.3 illustrate the cyclic arrangement of six helices (honeycomb cross-section) around a protruding central helix. Ring shaped networks of multiple chromophores (arrays) with controlled
inter-chromophore distances, are co-assembled with the DNA nanoscaffold by incorporating particular dye-modified oligonucleotides at selected positions. Multiple arrays of chromophores (triads) are arranged sequentially to facilitate a stepwise energy transfer cascade from the primary donor array (cyan) to the acceptor (red) through the intermediate dye array (orange, Figure 2.2).

**Figure 2.2.** Schematic display of the self-assembled 7-Helix Bundle (7HB) nanoscaffold that contains three distinct arrays of chromophores: the primary donors, the intermediate donors and the acceptor, represented by the cyan, orange and red rings, respectively. Upon excitation of the primary donor array, a step-wise energy transfer cascade is observed. The distance (along the helical axes) between the dyes in adjacent arrays is 3.5 base-pairs (the half base pair unit arises from the attachment of Cy3 to the sugar-phosphate backbone between two neighboring bases) or ~1.2 nm. The exact distances between the dyes must be calculated individually (described in greater detail in Figure S8) because of the differences in the attachment method, either to the base or to the DNA backbone.
The term “triad” corresponds to particular arrangements of the three chromophore arrays in the self-assembled DNA nanostructure. The four triads (T1-T4) used in this study contain dye ratios (primary donor : intermediate donor : acceptor) of 6:6:1, 6:3:1, 3:6:1 and 1:1:1, respectively. T1-T4 are schematically represented in Figure 2.3 with the corresponding positions of the dye molecules indicated on the DNA backbone. For each of the triads in Figure 2.3, the primary (Py, black sphere) and the intermediate donors (Cy3, dark green oval) are distributed among the 6 helices in the outer ring, and the acceptor (AF, pink sphere) is attached to the protruding central helix (as shown in Figure 2.2). The two arrays of the donor chromophores surround the acceptor chromophore with well-controlled inter-array distances.

In our design, the donor chromophores are attached to the DNA scaffold relatively rigidly. Py is incorporated into a 2′-deoxyuridine moiety through a rigid and short acetylene linker (substituting for the 5-H on the uridine base). Therefore, the aromatic ring of Py is expected to point towards the major groove of the corresponding DNA double helix without being fully exposed to the aqueous environment. Due to the steric of the bulky pyrene and the nearby base pairs, the plane of the aromatic pyrene ring more likely assumes a restricted orientational distribution with respect to the DNA helix. Cy3 is integrated within the sugar-phosphate backbone of the DNA oligonucleotide chain with a fixed orientation, linking the 5′-3′ ends of the two adjacent nucleotides. As a result its orientation is aligned with the backbone of the DNA double helix, exhibiting a well-defined angle with the helical axis. AF is linked to the DNA on the 5′ end through a flexible C5 linker (see Figures S2.2-S2.6). The attachment of AF to the DNA nanostructure is considered to be the most flexible among the three dyes. Although AF is highly charged
with the distribution of charge spread along the periphery of the conjugation, it is still possible to stack on the end of a nearby DNA helix in a similar manner as cyanine dyes such as Cy3 and Cy5. When these dyes are attached to the end of double helical DNA they interact with the DNA through base-pair stacking\textsuperscript{35} or may intercalate between base-pairs. Although a more detailed structural analysis is required to reveal the exact conformation, static fluorescence anisotropy measurements revealed that the three dyes attached to the 7HB DNA nanostructure displayed relatively high anisotropy: Py-0.27, Cy3-0.29, and AF-0.20. These values indicate that the electric dipoles of the dyes are not allowed to freely rotate relative to the DNA nanostructure, which is consistent with the rigid attachment of the dyes to the DNA.

The Förster distances ($R_0$) between the dyes (Table 2.1) are calculated from their spectral overlap, with the assumption that the dyes behave as point dipoles with a full range of orientations (i.e. $\kappa^2 = 2/3$).\textsuperscript{36} This assumption is not valid for our system as we have shown that the dipoles of the dyes cannot freely rotate relative to the DNA nanostructure. However, the calculated $R_0$ values should provide reasonable estimates of the distances necessary for significant energy transfer interactions.

The estimated distances between the dyes in adjacent arrays and among the dyes in the same array are listed in Figure S7 and S8. For T1 (Py:Cy3:AF = 6:6:1), the shortest distances between the Py and Cy3 dye molecules are calculated to be in the range of 2.1-2.7 nm, based on the underlying DNA nanostructure and the rigidity of the dye attachments. This distance range is significantly smaller than the calculated Förster distance for these two dyes (3.63 nm), and as a result efficient FRET can occur between neighboring Py and Cy3 pairs. The distance between the single AF acceptor and the 6 Cy3 dyes in the adjacent
Table 2.1. The calculated spectral overlap (J) and Förster distances (R₀) between the dyes.

The emission and excitation spectra of the dyes used for calculating the spectral overlap (J) and the quantum yields (QY) of the two donor dyes of Py and Cy3 are measured from individual dyes labeled on the 7HB structures (100 and 010 constructs). For simplicity, the orientation factor κ² was assumed to be 2/3 in the calculation for R₀.

<table>
<thead>
<tr>
<th></th>
<th>J (M⁻¹cm⁻¹nm⁴)</th>
<th>QY</th>
<th>κ²</th>
<th>R₀ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Py-Cy3</td>
<td>1.235x10¹⁵</td>
<td>0.1(Py)</td>
<td>0.66</td>
<td>3.63</td>
</tr>
<tr>
<td>Py-AF</td>
<td>5.91x10¹⁴</td>
<td>0.1(Py)</td>
<td>0.66</td>
<td>3.21</td>
</tr>
<tr>
<td>Cy3-AF</td>
<td>1.017x10¹⁶</td>
<td>0.23(Cy3)</td>
<td>0.66</td>
<td>5.93</td>
</tr>
</tbody>
</table>

array ranges from ~1.8 nm (shortest) to ~4.5 nm (longest), all of which are smaller than the Förster distance between Cy3 and AF (~ 5.93 nm), thus an efficient FRET between these dyes is also expected. The two shortest distances between the six Py dyes and the single AF acceptor are 2.4 and 2.6 nm, which are smaller than the calculated Förster distance between these two dyes. However, the FRET efficiency between Py and AF is expected to be lower than between the other pairs of dyes. A comparison of the Förster distances suggests that the most efficient energy transfer pathways in each triad are between the neighboring Py/Cy3 and Cy3/AF pairs.

The Py and Cy3 modified ssDNAs were created with a DNA synthesizer (ABI, 394 DNA/RNA Synthesizer, Applied Biosystems) using commercially available phosphoramidites (Glen Research), purified by reverse phase HPLC (Agilent 1200, equipped with both UV photodiode detector and automated fraction collector), and characterized using matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectroscopy (Applied Biosystem Voyager System 4320) (See Supporting
Figure 2.3. Schematic representation of each triad T1, T2, T3 and T4 (from left to right). The black spheres, dark green ovals and pink spheres represent Py, Cy3, and AF respectively. A simplified representation of each triad is also shown below the corresponding helical schematic, where the colored circles represent the presence of the dye molecules on the DNA helices.

Information for sequences and additional characterization details). AF modified and all other unmodified DNAs were purchased from Integrated DNA Technologies (www.idtdna.com) and purified by denaturing polyacrylamide gel electrophoresis (PAGE). The DNA nanoscaffold templated triads were constructed by mixing equimolar amounts of the appropriate DNA strands in 1×TAE buffer containing ~ 63.4 mM of Mg^{2+} and annealed from 90 °C to room temperature over 12 hrs. The formation of the desired 7HB structures was confirmed by nondenaturing polyacrylamide gel electrophoresis (Native PAGE, 5%) and the bands corresponding to the assembled structures were excised and extracted from the gels. The purified structures were again analyzed by Native PAGE and visualized with a fluorescence gel imager (Typhoon™ Trio multifunction imager,
Amersham Biosciences), as shown in Figure S1. For all four cases, the designed triads (T1–T4) migrated as a single, discrete band containing all the dye molecules as expected. The relative intensities of the bands in the fluorescence gel images also reflect the various ratios of dye molecules in particular structures.

A UV-Vis absorption spectrum of each purified structure was collected by a Jasco V-670 spectrophotometer (shown in Figure 2.4A and S2.10). The buffer solution was used to collect a background signal. Based on the extinction coefficients of the dyes, the concentration of each sample was found to be in the range of 50-80 nM. Each triad absorbs throughout the entire visible spectrum (350–700 nm) with distinct, well separated peaks at 400, 550, and 650 nm, characteristic of the Py, Cy3 and AF chromophores, respectively. The multi-dye absorption spectrum contains an essentially linear combination of the individual spectra, confirming that there are no ground state interactions between the chromophores in the self-assembled structures. The formation of intra-molecular excimers was not observed, even for those cases in which pairs of Py or Cy3 dyes within the same array are spaced very closely (~0.8 nm). The structures that contain particular numbers of chromophores are easily distinguished from the ratios of the characteristic absorbance peaks. The FRET process for each triad was investigated in detail using both steady state and time resolved fluorescence spectroscopy techniques. The fluorescence spectrum of a control structure (D1) that resembles T1 but contains an array of six Py only (with no Cy3 nor AF) exhibited emission features characteristic of a monomeric Py (Figure 2.4B, black trace).

When T1 (with dye ratio of 6:6:1) is excited at 380 nm (the wavelength of Py absorption), a drastic quenching of Py emission at 438 nm compared to that of D1, and
strong emission peaks at 566 and 668 nm that are characteristic of Cy3 and AF, respectively, are observed (Figure 2.4B, red trace). From this data, the FRET efficiency, calculated from the decrease in Py emission, is estimated to be ~ 90%. The observed emission from AF upon photo-excitation of Py is most likely due to the stepwise energy transfer from the Py array to AF through the intermediate Cy3 array. This is confirmed by the analysis of a dyad control sample (D2) that is structurally similar to T1, containing 6 Py and 1 AF but lacking the intermediate Cy3 array. D2 exhibited a very weak emission peak at 668 nm, with the same 380 nm excitation (shown in Figure S2.11B). Although the direct energy transfer from Py to AF cannot be completely ignored, its occurrence is significantly reduced in the presence of the Cy3 array. This is primarily because Cy3 has larger spectral overlap with both Py and AF and is positioned more closely to Py and AF than they are to each other.

The efficiency of energy transfer is strongly influenced by the ratio of Py to Cy3. For example, T1, with a Py: Cy3 ratio of 6:6 (=1:1), exhibits a remarkable FRET efficiency up to ~90%. In the case of T2, with a Py: Cy3 ratio of 6:3 (=2:1), the FRET efficiency is drastically reduced to ~30%. This may be because of the decrease in the number of Cy3 in this triad; in the moments after excitation, only half of the excited Py can find a proximal Cy3 to relay the energy to, while the other half of Py either have to relay the energy to a more distant Cy3 via a slower energy transfer or relax back to the ground state by fluorescence emission or non-radiative pathways. However, for T3, reversing the Py: Cy3 ratio to 3:6 (=1:2) restores the FRET efficiency to ~90%, similar to the T1 triad. This implies that the FRET efficiency reaches a maximum value when the number of intermediate donor Cy3 molecules is maximized. Reducing the number of primary donor
Py molecules does not have any considerable effects on the energy transfer efficiency, but only reduces the initial amount of light absorbed. It is important to note that the FRET efficiency in T4 (Py:CY3:AF = 1:1:1) is ~70%, lower than that for T1, even though both triads have the same Py:CY3 ratio of 1:1 and the same distance (shortest) between Py and Cy3. This difference in the FRET efficiency indicates that the presence of multiple Py and Cy3 pairs in T1 provides several energy transfer pathways. Therefore, T1 exhibits significantly higher energy transfer efficiencies than T4, which only contains a single pathway.

It is important to acknowledge that the expected FRET efficiencies (based on the Förster distances) are larger than the measured values, possibly because of orientation effects. The allowed angles and relative orientations between the dipole moments of the chromophores may not be optimized for the most efficient energy transfer due to the relatively rigid attachment of the dyes to the DNA nanostructures. The number of possible orientations of the dyes is limited by their individual conformation with respect to the helical axis of the DNA and by their position within the DNA helix. In addition, because of the anti-parallel alignment of the complementary strands within each DNA helix, two dyes labeled on the same DNA strand may assume a 180 degree relative orientation when they are separated by a crossover point. These effects can dramatically decrease the value of $\kappa^2$ (the orientation effect), thus reducing the observed FRET efficiency.

In addition to evaluating the FRET efficiency of each energy transfer step, the light harvesting ability of each triad is determined by evaluating the so called “antenna” effect, $7(a)$ which is defined as the ratio of the fluorescence intensity of the acceptor upon excitation of the donor to that of the direct excitation of the acceptor. The antenna effect indicates an
overall increase or decrease in the acceptor emission resulting from a change in the donor, whereas the efficiency of energy transfer specifies the increase or decrease in donor emission with a change in the acceptor. The overall antenna effect (AE1) is calculated using the following equation: 

\[ AE1 = \frac{I_{AF,380\text{nm}}}{I_{AF,620\text{nm}}} \]

where \( I_{AF,380\text{nm}} \) and \( I_{AF,620\text{nm}} \) are the fluorescence intensities of AF upon excitation of the primary donor (Py) at 380 nm and the direct excitation of AF at 620 nm. The antenna effect for the second step (AE2) is calculated according to the following equation: 

\[ AE2 = \frac{I_{AF,500\text{nm}}}{I_{AF,620\text{nm}}} \]

that compares the fluorescence intensity of AF upon excitation of the intermediate donor Cy3 at 500 nm to the direct excitation of AF.

The overall antenna effect for T1 is ~85 %, indicating that the emission of the AF acceptor, after indirect excitation through step wise FRET energy transfer from the Py donors, is only slightly lower than the emission when the acceptor is directly excited. This result demonstrates the light-harvesting capability of our system is quite efficient. The antenna effect was negligible for a control construct which contained only the acceptor without the primary and intermediate donor arrays, because 380 nm light is not efficiently absorbed by the acceptor.

The antenna effects for triads T2, T3 and T4 were analyzed to determine the effect of the number of donors on the light harvesting ability. T2 and T3 with Py:Cy3:AF ratios of 6:3:1 and 3:6:1 demonstrate comparable antenna effects of ~43 % and ~47 %, respectively, which is about half of the antenna effect of T1. T2, with 6 primary donors, initially absorbs the same amount of light as T1, however, T2 only has 3 intermediate donors to relay the energy to the final acceptor (compared to 6 intermediated donors in T1)
and the result is a decrease in the antenna effect. While T3 has 6 intermediate donors to transfer the energy to AF, the initial amount of light absorbed by the 3 Py chromophores is approximately half that of T1, producing a similar reduction in the antenna effect as T2. The antenna effect is only ~16% for T4, which has a single Py and Cy3 donor molecule. Although the relative number of Py to Cy3 is the same for T1 and T4, the initial absorption of energy by a single Py molecule in T4 results in a significant decrease in the antenna effect.

By definition, the antenna effect is an empirical measure of the light harvesting efficiency of a system. It is proportional to the product of the efficiencies of each energy transfer step, to the ratio of the extinction coefficients of the donor and the acceptor at their excitation wavelengths and to the ratio of the number of donor to acceptor dyes (i.e. the ratio of the excitation photons absorbed at the different wavelengths). The greater the number of primary donors to absorb light in the first step of the energy cascade, the greater the energy available for the downstream transfer of energy; in addition, the higher the efficiency of the energy transfer between dyes, the more light that will be emitted by the final acceptor of the relay. T1 has 6 Py and 6 Cy3; T2 has 3 Py and 6 Cy3; T3 has 6 Py and 3 Cy3; and T4 has 1 Py and 1 Cy3, and although the initial energy gain by T1 and T3 are the same, the intermediate transfer of energy through Cy3 is not equal for the two triads. Therefore, we can predict the overall antenna effect will exhibit a trend of T1>T2≈T3>T4, with an approximate ratio of 6:3:3:1. This trend was experimentally confirmed (Table 2.2).

The antenna effect for the second step of the energy transfer cascade can be evaluated by the direct excitation (500 nm) of the secondary donor (Cy3) (Table 2.2). The predicted trend for antenna effect 2 (T1≈T3>T2>T4) is based on the number of Cy3
chromophores in each triad, which exhibit a ratio of 6:6:3:1. The experimental results are in reasonable agreement with the predicted values. Overall, the results indicate that the excitation energy can be efficiently funneled in a stepwise manner to the acceptor core by peripheral donor excitation.

Figure 2.4. (A) Absorption spectrum of T1 (additional absorption spectra are shown in Figure S10). (B) Normalized emission spectra of D1 and T1–T4, all with excitation at 380 nm. For all samples, the emission spectra were corrected by the PMT detector spectral response file within the instrument software, and the normalization was done by dividing the emission spectra by the absorption value of each individual sample at 380 nm. The original raw absorption and emission data are shown in Figure S2.10 and S2.11.

Detailed time resolved fluorescence analyses were performed on each triad to elucidate the kinetics and dynamics of the cascading FRET processes. First, time-correlated single photon counting (TCSPC) was used for fluorescence decay analysis (Figure 2.5). The decay analysis of the individual Py, Cy3, and AF constructs revealed bi-exponential decay profiles with lifetimes of 0.35 ns and 3.2 ns for Py, 0.45 ns and 2.1 ns for Cy3, and 0.34 ns and 1.3 ns for AF (see Figure S2.20). Figure 5 contains the decay profiles of Py in
Table 2.2. The measured FRET efficiency and antenna effect for each triad, where the FRET efficiency is calculated by the quenching of Py emission compared to the D1 sample. Antenna effects 1 and 2 are obtained by comparing the emission of AF by excitation at Py or Cy3 to the emission of AF by direct excitation.

<table>
<thead>
<tr>
<th>System</th>
<th>FRET efficiency (%)</th>
<th>Antenna effect 1 (%)</th>
<th>Antenna effect 2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (6:6:1)</td>
<td>90</td>
<td>85</td>
<td>93</td>
</tr>
<tr>
<td>T2 (6:3:1)</td>
<td>30</td>
<td>43</td>
<td>39</td>
</tr>
<tr>
<td>T3 (3:6:1)</td>
<td>90</td>
<td>47</td>
<td>89</td>
</tr>
<tr>
<td>T4 (1:1:1)</td>
<td>70</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

D1 and T1–T4, monitored at 460 nm (using λ<sub>ex</sub> = 370 nm). The profiles clearly show that the decay of Py becomes faster in the presence of Cy3 and AF as the number of Cy3 molecules is increased, following the trend revealed by the steady state FRET efficiency measurements (T1≈T3>T4>T2). The significant acceleration of the decay dynamics of the Py donor in the presence of the acceptors provides clear evidence of FRET.

Further evidence of a step-wise energy transfer process is provided by the rise in the emission of Cy3 and AF, monitored at 560 nm and 660 nm, respectively, upon excitation of Py at 370 nm (Figure 2.6). At the initial time scale (~10 ps to ~300 ps) the observed rise component for Cy3 and AF in T1-T4 coincides with the decay of Py, representing an increasing excited state population of both chromophores through energy transfer from the photo-excitation of Py. In addition, the rise and decay of AF is slower than Cy3 for all four cases, indicating that AF is involved in the final step of the step-wise energy transfer relay. For T4, the rise and decay of AF follows Cy3 very closely, probably
Figure 2.5. Fluorescence decay profile of ssDNA (5’-ATTATAPyATAGCGTCGTGCG ACTGGCATGTGATAC-3’), D1 and T1–T4 monitored at 460 nm ($\lambda_{ex} = 370$ nm).

because of the particular arrangement of the dyes; the distance between Cy3 and AF is similar to the distance between Py and AF with the potential for one step energy transfer from Py to Cy3 and Py to AF.

The TCSPC instrument response time is on the order of ~40 ps, and thus very short lifetimes cannot be obtained reliably. To improve the temporal resolution, a streak camera was utilized. Emission decay data from a spectral range of 450 nm to 680 nm, with excitation at 370 nm, was obtained for each triad sample. The instrument simultaneously provided high temporal resolution (~2 ps) and high spectral resolution (~5 nm). The 3D data for a typical sample is shown in Figure S2.14 and additional decay profiles at various wavelengths are shown in Figures S2.16-S2.19. Global life-time analyses was performed using the same set of lifetimes for the decay at different wavelengths; this determines the
spectral dependence of the decay amplitudes associated with each lifetime, also known as the decay associated spectra (DAS) (shown in Figure 2.7). DAS offers better insight into the mechanisms of multistep energy transfer processes than emission decays monitored at a single wavelength. T4 can be adequately described by 3 lifetime components, while the other three samples T1-T3 require an additional component.

The lifetimes for the T4 fluorescence decay profile (with a dye ratio of 1:1:1) are 40 ps, 0.43 ns and 2.4 ns. The corresponding DAS spectrum (Figure 2.7) for the component with the shortest lifetime (40 ps) shows a positive amplitude in the Py emission region (450-540 nm) and a negative amplitude in the Cy3 emission region (540-650 nm). These spectral features indicate that the 40 ps lifetime component corresponds to the decay of the Py excited state population with the simultaneous buildup of the Cy3 excited state population, resulting from the energy transfer from Py to Cy3 that occurs within this short lifetime. The 40 ps lifetime data also shows small negative amplitude in the AF spectral range, indicating the population buildup of the excited state of AF may occur in a similar time scale as Cy3. This may suggest a very fast energy transfer rate from Cy3 to AF, possibly with a small amount of direct energy transfer from Py to AF (Figure S2.23). The DAS spectra of the components for the 0.43 ns and 2.4 ns lifetimes contain one main, intense positive band in the Cy3 emission region, which can be attributed to the decay of the excited state population of Cy3 resulting from the energy transfer from Cy3 to AF and the decay of Cy3 to its ground state. However, the small positive (non-zero) amplitudes at 450 nm and 660 nm for these two components also indicate minor contributions from Py and AF decays in the longer lifetime scale, consistent with the results obtained by the TCSPC.
Figure 2.6. Time resolved emission of T1 (A), T2 (B), T3 (C) and T4 (D) monitored at 460 nm (Py decay, black), 560 nm (Cy3 decay, red) and 660 nm (AF decay, blue).

The fluorescence decay profile for T3 (with dye ratio of 3:6:1) exhibits a four-exponential decay with lifetimes of 32 ps, 225 ps, 522 ps and 2.7 ns. The two shortest lifetimes display positive amplitudes in the Py spectral region and negative amplitudes in the Cy3 and AF spectral region, which represent the Cy3 population build up and the simultaneous decay of Py; the two longer lifetimes exhibit positive amplitudes in the Cy3 and AF spectral region, representing the decay of the two acceptor dyes. The occurrence of two short lifetime components also indicates that both the short (~2.1 nm) and long (~3.5 nm) distance energy transfer processes between Py and Cy3 occur in T3, corresponding to the two lifetimes, 32 ps and 225 ps, respectively. Of the two shorter lifetime components,
Figure 2.7. DAS of (A) T1 (6:6:1), (B) T2 (6:3:1), (C) T3 (3:6:1), and (D) T4 (1:1:1), where the numbers in the parenthesis indicate the ratio of the three dyes in each structure. The shortest component has a far more negative amplitude than the second shortest component, which indicates that the short range pathway dominates in the energy transfer process. This is probably because of the smaller ratio of Py to Cy3 in this sample (3:6), i.e. although every excited Py has at least two Cy3 to transfer energy to, the Cy3 that is the closest to Py likely wins the kinetic competition.

Triads T1 and T2, with a larger number of primary donor molecules, also display 4 lifetimes: 30 ps, 159 ps, 533 ps, and 2 ns for T1, and 41 ps, 276 ps, 564 ps, and 2.1 ns for T2. Similar to T3, the two shortest lifetime components in the DAS spectra for T1 and T2 both exhibit positive amplitudes in the Py emission region due to the excited state decay.
of Py, and negative amplitudes in the Cy3 and AF emission region due to the simultaneous population increase of the excited state of Cy3 and AF.

The long range energy transfer pathway (with lifetimes of 160-270 ps) has a slightly more important role in T1 (Py: Cy3 = 6:6, Figure 2.7A) than in T3 (Py: Cy3 = 3:6, Figure 2.7C). It is expected that as more Py molecules are available for excitation, the probability of the long range energy transfer process increases. However, in T2 (Py: Cy3 = 6:3) the longer range energy transfer component shows a larger amplitude than the shortest lifetime component (Figure 2.7B). One possible explanation for this is that every Py in T2 has the same probability of being excited, however, only half of the excited chromophores can find a nearby Cy3 to accept energy, while the other half would have to transfer energy via the longer distance pathway. At the same time, the Py in T2 that have nearby Cy3 also have a probability of transferring the energy through the longer distance pathway, as seen in the case of T1 and T3.

It should be noted that the DAS associated with the ~500 ps lifetime, which should reflect the energy transfer from Cy3 to AF, does not exhibit the expected signature shape as is seen in the ~40 ps DAS. It is likely that the negative amplitude in the AF spectral region is superimposed on the positive signal from Cy3 emission with the same time constant. However, comparison of the ~500 ps DAS with the ~2 ns DAS reveals an obvious amplitude increase in the AF emission in the 2 ns DAS (Figure S2.24). This confirms that the relative excited state population of AF to Cy3 has increased in the later time, and provides evidence that the energy transfer from Cy3 to AF occurs within 500 ps.
2.5. Conclusion

We have employed DNA nanotechnology to create a structurally well-defined, DNA templated, artificial light harvesting antenna. Three distinct chromophore arrays were arranged into several triad configurations with precise inter-chromophore distance and well-defined donor-acceptor ratios. Steady state and time resolved fluorescence analyses revealed that efficient, step-wise FRET from a primary donor array to an acceptor through an intermediate donor array occurs upon excitation of the primary donor. Although multiple energy transfer pathways are possible in each multi-dye array, unidirectional energy transfer to the final acceptor was always observed. In addition, the relative donor-acceptor ratio had a profound effect on the efficiency of energy transfer and the antenna effect.

This study undoubtedly demonstrates that DNA based nanoscaffolds are excellent platforms to organize arrays of chromophores with precise control of each structural element, and provide the flexibility necessary to test several factors governing the antenna effect, such as the molar ratio of the dyes, the ratio of the extinction coefficients of the donor and acceptor, the quantum yields and the spectral overlap. It may ultimately provide essential guidelines for the future design of artificial light-harvesting systems. For example, for an efficient light harvesting system the antenna dyes should cover a broad absorption range, have high extinction coefficients, high quantum yields and broad emission spectra. The dyes used here are far from optimized to achieve the best light harvesting efficiency. Other blue absorbing dyes with higher extinction coefficients and higher quantum yields may be considered in the future, and more complex design could be employed to further improve the light harvesting ability and energy transfer efficiency.
2.6. References


Chapter 3
Reengineering the Optical Absorption Cross-section of Photosynthetic Reaction Centers


3.1. Abstract

Engineered cysteine residues near the primary electron donor (P) of the reaction center from the purple photosynthetic bacterium *Rhodobacter sphaeroides* were covalently conjugated to each of several dye molecules in order to explore the geometric design and spectral requirements for energy transfer between an artificial antenna system and the reaction center. An average of 2.5 fluorescent dye molecules were attached at specific locations near P. The enhanced absorbance cross-section afforded by conjugation of Alexa Fluor 660 dyes resulted in a 2.2 fold increase in the formation of reaction center charge separated state upon intensity-limited excitation at 650 nm. The effective increase in absorbance cross-section resulting from the conjugation of two other dyes, Alexa Fluor 647 and Alexa Fluor 750, was also investigated. The key parameters that dictate the efficiency of dye-to-reaction center energy transfer and subsequent charge separation were examined using both steady-state and time-resolved fluorescence spectroscopy as well as transient absorbance spectroscopy techniques. An understanding of these parameters is an important first step towards developing more complex model light harvesting systems integrated with reaction centers.
3.2. Introduction

One of the most fascinating phenomena in nature is the primary solar energy conversion event in photosynthesis. Photosynthetic organisms employ a light-harvesting antenna network to collect photons and transfer their energy to the reaction center, where the energy is used to power a series of electron transfer reactions with near unity quantum yield. The geometry and spectral properties of the light-harvesting systems used by different organisms are quite varied, depending on environmental conditions and needs. Both to further our fundamental understanding of light harvesting and to enable the engineering of artificial photonic systems, it would be useful to develop platforms in which model complexes of pigments and charge separation elements can be assembled in a spatially defined manner.

Quantum dots and organic fluorophores have been conjugated previously with reaction centers (RCs) and in some cases used to enhance the absorbance cross-section of the photosynthetic RC by absorbing light in spectral regions to the blue of the natural reaction center absorbance and then transferring energy to the reaction center initial electron donor. However, a more detailed understanding of how the specific spectral and excited-state properties of the absorbers as well as the relative geometry of the different components contribute to the overall performance of light-harvesting systems would be beneficial. Here, a genetically modified RC (Figure 3.1A) is used in conjunction with commercially available fluorescent dye molecules to develop a geometrically defined system for systematically studying the effects of pigment spectrum, attachment point, and fluorescence lifetime on the energy-transfer efficiency to the reaction center. This study
should help define some of the parameters important for designing novel molecular photonic devices.

3.3. Methods and Materials

3.3.1. Reaction Center Mutations: The RC we used here contains a total of eight mutations relative to the wild type. Five of the mutations served to replace the five wild type cysteines with serine and alanine, and the remaining three mutations introduced cysteines at the points of interest. The eight mutations are as follows: (H)C156A, (H)C234S, (L)C92S, (L)C108S, (L)C247S, (L)E72C, (L)N274C and (M)E100C. The RC contains a six histidine tag at the C-terminus of the H subunit to facilitate purification with a Ni-sepharose affinity column.4

3.3.2. RC Isolation and Purification: RCs were isolated from a mutant which was derived from *R. sphaeroides* 2.4.1 using a modification of a procedure previously published.4,5 In short, the cells were grown at 30 °C in 2 L of modified LB medium containing 810 µM MgSO₄, 510 µM CaCl₂, and 4 mM NaCl, using 2 L Erlenmeyer flasks, shaken at 250 rpm. After 3.5 days the cells were centrifuged at 9000 g and resuspended overnight in 50 mM phosphate buffer (pH 8) containing 150 mM NaCl. The cells were then lysed using a French Press followed by addition of small amount of DNase. Unbroken cells were removed via centrifugation at 9000 g and the remaining supernatant was treated with imidazole (final concentration 5 mM) and N, N-Dimethyldodecylamine N-oxide (LDAO, final concentration 0.6% by weight). After 15 min of incubation, the solution was centrifuged at 14000 g followed by Ni-sepharose purification of the RC from the supernatant. The following paragraph lists the buffers used in the Ni-sepharose column purification.
Wash buffer: 50 mM phosphate buffer, pH 8, 0.1% LDAO, 150 mM NaCl, 5 mM imidazole
Elution buffer: 50 mM phosphate buffer, pH 8, 0.1% LDAO, 150 mM NaCl, 100 mM imidazole

Column wash buffer: 50 mM phosphate buffer, pH 8, 0.1% LDAO, 300 mM NaCl, 250 mM imidazole. The eluted protein was further purified by dialysis against 15 mM Tris, pH 8, 150 mM NaCl, 1 mM EDTA containing 0.025% LDAO) overnight to remove excess imidazole and LDAO. The concentration of the purified RC was measured using absorbance at 804 nm (extinction coefficient ~288000 M⁻¹cm⁻¹).³c

### 3.3.3. Quinone Removal Procedure:

A concentrated RC solution was diluted such that the OD₈₀₄ was ~0.1 (corresponding to 0.37 µM) at a final volume of 200-250 mL in high-concentration LDAO buffer (10 mM Tris-HCl, 4% LDAO, 10 mM 1,10-phenanthroline, pH 8). This solution was stirred for 2 hrs at 25°C, and applied to a Di-Ethyl-Amino-Ethyl (DEAE) column at 4 mL/min loading speed, followed by washing with high LDAO buffer for 2 hrs and a flow rate 2 mL/min. The column was then washed with a buffer containing a low concentration of LDAO (15 mM Tris-HCl, 0.025% LDAO, 1 mM EDTA, pH 8) for 1 hr and then the RC was eluted with 15 mM Tris-HCl, 0.25 M NaCl, 0.1% was, 1 mM EDTA, pH 8 followed by overnight dialysis. The quinone-depleted RC (Q₆₅₃RC) was used in transient absorbance experiments to observe the trapped charge-separated state.

### 3.3.4. RC-Dye Conjugation Procedure:

1 mg of dye (AF647, AF660 or AF750) functionalized with a maleimide group (Invitrogen) was dissolved in DMSO to make a 15 mM solution. A solution of 50-70 µM RC in 1× PBS (pH 7.4, containing 0.025% LDAO) was first treated with 10-fold excess of 50 mM Tris(2-carboxyethyl)phosphine
hydrochloride (TCEP-HCl; Thermo Scientific) to reduce possible disulfide bonds, and the protein was washed four times with the 1× PBS buffer mentioned above using an Amicon centrifugal filter (50 kD molecular weight cut-off). The TCEP-treated RC was added to the dye solution with a RC-dye molar ratio of 1:15 and incubated overnight at 4 °C. The maleimide group of the dye is expected to link to thiol of cysteines on the RC surface through a coupling reaction. Excess glutathione (1.5 fold to the dye) was added to consume the unreacted thiol-reactive dye molecules. The mixture was then washed three times using Amicon centrifugal filter (50 kD molecular weight cut-off) with 1× PBS buffer containing 0.025% LDAO to remove excess dye molecules. The sample was further purified by Ni-sepharose chromatography and dialyzed overnight against tris buffer (15 mM Tris-HCl, pH 8, 0.025% LDAO, 150 mM NaCl, 1 mM EDTA).

3.3.5. BSA-Dye Conjugation and Purification: A 50 μM solution of BSA (Sigma) in 1×PBS (pH 7.4, 0.025% LDAO) was treated with 50 mM TCEP-HCl (5 fold molar excess) and washed with 1× PBS buffer mentioned above using an Amicon centrifugal filter (30 kD molecular weight cut-off), and then the RC was added to the dye solution with a BSA-dye molar ratio of 1:15. The reaction mixture was kept overnight at 4°C. Excess glutathione was added to consume the unreacted dye molecules. The mixture was then washed three times using Amicon centrifugal tube (30 kD molecular weight cut-off filter) with 1×PBS buffer containing 0.025% LDAO to remove excess dye molecules. The sample was then dialyzed overnight against tris buffer (15 mM Tris-HCl, pH 8, 0.025% LDAO, 150 mM NaCl, 1 mM EDTA). The concentration of the purified BSA was measured using absorbance at 279 nm (extinction coefficient ~ 44308 M⁻¹cm⁻¹).
3.3.6. MALDI-TOF Procedure: A mixture of formic acid/water/isopropyl alcohol (IPA) (3:1:2) was prepared 4 hrs in advance prior to the sample preparation. A saturated solution of α-cyano-4-hydroxycinnamic acid (4HCCA) was then prepared in the above mixture and centrifuged at 14000 g for 6 min to remove any matrix micro-aggregates. The supernatant was transferred to a fresh tube and named Matrix 2. A 20 μM solution of RC was diluted to 5 μM with 0.1% trifluoroacetic acid (TFA) (v/v) followed by dilution in matrix 2 (1:5, v/v). Next a saturated solution of 4HCCA in 1:1 (v/v) mixture of TFA aq. 0.2%/acetonitrile was prepared and centrifuged to remove any matrix aggregates. The supernatant was transferred into a new tube, diluted 4-fold with IPA, and named Matrix 1. 20 μL of Matrix 1 was applied over a clean sample plate (5 cm × 5 cm), and allowed to partially dry. When the plate was almost dry, the matrix was wiped off the plate using a Kimwipe tissue, leaving behind a faint layer of 4HCCA, which was only visible as a yellowish reflection when the plate was angled towards the light. Following this, 0.5 μL of protein diluted in Matrix 2 was deposited onto the thin layer of 4HCCA. After drying the plate, MALDI was carried out in an Applied Biosystem Voyager System 4320 with an accelerating voltage of 25000 V.

3.3.7. Absorption and Fluorescence Spectroscopy: Absorption spectra were measured using a quartz cuvette (1 cm path length) in a Jasco V-670 spectrophotometer. Steady state fluorescence spectra were obtained in a nanolog fluorometer (Horiba Jobin Yvon), using a quartz cell (1 cm path length), and all emission spectra were corrected for the wavelength dependence of the detection system response. Fluorescence life-time decay measurements were analyzed by time-correlated single-photon-counting as described below.
3.3.8. Light-Minus-Dark Experiment: The light-minus-dark spectra were obtained by subtracting the absorbance spectrum of a sample taken in the dark from the spectrum of the same sample exposed to a continuous, actinic light source centered at 650 nm (bandwidth ~10 nm) that had illuminated the sample for 3 minutes prior to measurement. The path of the actinic beam was perpendicular to the path of the probe light from the UV-Vis absorbance spectrophotometer. The excitation light at 650 nm was obtained using a white light source (Dolan-Jenner MH-100 Metal Halide Fiber Optic Illuminator) passed through two filters (RG610 and IF650; 10 nm band pass). For all measurements, samples contained a 50-fold excess of 1,10-phenanthroline compared to the RC concentration.

3.3.9. Time-Correlated Single-Photon Counting Kinetic Measurement: The excitation source was a fiber supercontinuum laser (Fianium SC450) operated at 20 MHz. The laser output was sent through an Acousto-Optical Tunable Filer (Fianium AOTF) to obtain excitation pulses at wavelengths of 600 nm and 710 nm. Fluorescence emission was collected at a 90° geometry setting and detected using a double-grating monochromator (Jobin-Yvon, Gemini-180) and a microchannel plate photomultiplier tube (Hamamatsu R3809U-50). The polarization of the emission was 54.7° relative to that of the excitation. Data acquisition was done using a single photon counting card (Becker-Hickl, SPC-830). The typical IRF (instrument response function) had a FWHM (full width half maxima) of 50 ps, measured from the light scattered from sample at the excitation wavelength. The data were fitted using a locally written software package, ASUFIT, to a sum of exponential decay terms either globally (at many wavelengths simultaneously) or at a single wavelength.
3.3.10. Calculation of FRET Efficiency and Average Lifetime of Dye Molecules: FRET efficiencies \( E \) were calculated according to the following equation:

\[
E = 1 - \frac{I_{DA}/A_{DA}}{I_D/A_D}
\]

Where \( I_{DA} \) and \( I_D \) are the integrated area of donor fluorescence emissions with and without acceptors. \( A_{DA} \) and \( A_D \) are the absorbance of donor at excitation wavelength with and without acceptors.

Average lifetimes were calculated using the following equation.

\[
\tau_{ave} = \frac{\sum_i A_i \tau_i}{\sum_i A_i}
\]

Where \( A_i \) and \( \tau_i \) are the amplitude and the lifetime components.

3.3.11. Time-Resolved Ultrafast Transient Absorption Spectroscopy: Transient absorbance measurements were performed using a broadband and narrowband pump-probe system as described previously.\(^6\) Laser pulses of 100 fs at 800 nm were generated from a regenerative amplifier system (Tsunami and Spitfire, Spectra-Physics) operated at 1 kHz. Part of the beam was used to pump an optical parametric amplifier (IR OPA, Spectra-Physics) to generate excitation pulses at 650 nm. The white-light probe pulses were generated by focusing part of the 800-nm beam onto a 3 nm sapphire plate and detected using a CCD camera (DU420, Andor Technology) for probing kinetics over a 140-nm wavelength window simultaneously. The collected data had a 2.3 nm spectral resolution. In some measurements, kinetics at a specific wavelength were recorded using a photodiode for higher signal-to-noise ratio. All time-resolved experiments were performed at room temperature. The absorbance changes as a function of time \( (t) \) and probe wavelength \( (\lambda) \).
were fit globally to a multiple exponential model using locally written software, ASUFIT. The instrument response time function was fitted to a Gaussian curve (FWHM of 150 fs).

3.3.12. Cytochrome c Oxidation Experiment: Cytochrome c oxidation kinetic spectra were obtained by measuring the absorbance change at 550 nm in the presence of a 650 nm or 800 nm excitation beam. The excitation light centered at 800 nm was generated by passing white light through an 800 nm band pass filter (FB800-40, FWHM 40 nm). Light intensity at 650 nm was 5.8 fold higher than that at 800 nm. The sample contained 1 μM dye-modified RC (RC-AF647 or RC-AF660), 100 μM decylubiquinone (Sigma-Aldrich) and 10 μM reduced bovine heart cytochrome c (Sigma-Aldrich) in tris buffer (15 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 0.025% LDAO, pH 8). Cytochrome c was reduced by using a published procedure. In short, 1 mL of cytochrome c solution (1.2 mM) was mixed with a 10-fold molar excess of sodium ascorbate (600 mM stock solution) in 10 mM sodium phosphate buffer (pH 6.9) and agitated for 3 hrs at 4°C. The solution was then desalted using a Nap-25 column (GE Healthcare) to remove excess sodium ascorbate by washing with 10 mM sodium phosphate (pH 6.9) buffer, followed by tris buffer. The concentration of the reduced cytochrome c was calculated by measuring the absorbance at 550 nm (extinction coefficient 28000 M⁻¹cm⁻¹). Two different excitation lights were used, 650 nm and 800 nm.

3.4. Results and discussion

Here, a mutant of the *Rhodobacter sphaeroides* 2.4.1 RC (PDB 2J8C) is used that contains three specific and unique cysteine residues near the primary electron donor, P. These Cys residues have been conjugated to several commercially available fluorescent dye molecules with different spectral properties. Using this system, the interplay between
Figure 3.1. (A) Structure of the RC from the purple bacterium, *Rhodobacter sphaeroides* 2.4.1 (PDB 2J8C). The three protein subunits, M, L and H and the cofactors (except carotenoid) are shown. Three unique Cys residues have been introduced (shown in red) and their relative distances are given. There are two Cys residues on the L subunit and one on the M subunit. (B) Normalized absorption spectra of the RC (black), AF660 (green) and AF750 (red). A normalized absorption spectrum of AF647 is not shown because it is similar to AF660.

absorbance cross-section, spectral breadth of light harvesting, excited state lifetime and energy transfer efficiency can be explored.

The structure of the RC complex is shown in Figure 3.1A, which consists of three polypeptide subunits H, M and L. The L and M subunits are associated with ten cofactors: a dimer of bacteriochlorophylls denoted as P, two bacteriochlorophylls (B_A and B_B), two bacteriopheophytins (H_A and H_B), two molecules of ubiquinone-10 (Q_A and Q_B), one carotenoid and one nonheme iron atom (Fe^{2+}). P is the primary electron donor and upon excitation, it transfers an electron to Q_A via B_A and H_A, forming a long-lived charge
separated state $P^+Q_A^-$. Eventually electron transfer occurs from the reduced primary quinone to the secondary quinone $Q_B$ forming $P^+'Q_B^-$. There are three distinct spectral bands between 700 nm and 900 nm in the RC absorption spectrum (Figure 3.1B, black curve), which predominantly represent $H_A$ and $H_B$ (760 nm), $B_A$ and $B_B$ (804 nm), and $P$ (860 nm), respectively.⁹

The genetically modified RC protein used in this study contains three Cys residues (M100C, L72C and L274C) located on the surface of M and L subunits respectively, close to the P site. All the other Cys residues were replaced with either serine or alanine.⁴ Fluorophores were covalently conjugated to these three surface Cys residues via reactive maleimide groups (for details of the sample preparation, purification and characterization see supporting information). The cysteines are situated more than 3.5 nm from one another to avoid the possibility of intramolecular disulfide bond formation and also to prevent direct interactions between the attached fluorophores. The distances between each Cys and P range from 3.0 to 3.7 nm. By selecting different dye molecules the overall absorption cross section of the assembled system can be tuned over a broad range (Figure 3.1B).¹⁰

The three dye molecules used in this study are Alexa Fluor 647 (AF647, $\lambda_{\text{max,abs}} = 649$ nm, $\lambda_{\text{max,em}} = 667$ nm, fluorescence quantum yield = 0.33), Alexa Fluor 660 (AF660, $\lambda_{\text{max,abs}} = 660$ nm, $\lambda_{\text{max,em}} = 690$ nm, fluorescence quantum yield = 0.37) and Alexa Fluor 750 (AF750, $\lambda_{\text{max,abs}} = 752$ nm, $\lambda_{\text{max,em}} = 780$ nm, fluorescence quantum yield = 0.12). The fluorophores were chosen in such a way that they substantially increase the absorbance cross section in the spectral regions where the absorbance of the RC is low, and there is significant spectral overlap between the emission spectra of the dye and the absorbance of the RC pigments.
To better understand the spectral and kinetic properties of the Alexa Fluor dyes in a protein environment without an energy acceptor, the dye molecules were conjugated to a spectrally inactive protein, bovine serum albumin (BSA) using the same maleimide chemistry used for RC-dye conjugation. These samples were used as controls in the spectroscopic measurements. A 4-nm red-shift in the absorbance and a 7-nm red-shift in the fluorescence of the AF660 dye were observed when it was attached to a surface-exposed Cys of BSA (average dye/BSA ratio 0.8), compared to the free dye in solution. Similar spectral shifts were observed for BSA-AF647 conjugates (average dye/BSA ratio 0.8) and BSA-AF750 conjugates (average dye/BSA ratio 0.6) (Figures S3.2-S3.4). The shift apparently reflects the influence of the protein environment on the spectral properties of the dye molecules. A comparison between fluorescence quantum yield of free dye and the BSA conjugated dyes is shown in Table 3.1.

The three dyes (AF647, AF660 and AF750) were conjugated separately to the RC with dye-to-RC ratios of 2.5, 2.6 and 2.2, respectively. The covalent conjugation of AF660 to the RC was confirmed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry using α-cyano-4-hydroxycinnamic acid as matrix.\textsuperscript{3a,11} The RC shows three distinct peaks corresponding to its three protein subunits (H, L and M), whereas the RC-AF660 conjugate exhibits two additional peaks associated with the L-subunit and one additional peak associated with the M-subunit, each with a 840-920 Da mass shift. This result confirms that the conjugation of the dye molecule to the RC protein was selective and site-specific (Figure 3.2B and S3.1).
Figure 3.2. (A) Absorption spectra of quinone-depleted RCs ($Q_{\text{del}}$RC, purple) and AF660 conjugated quinone-depleted RCs with dye to RC ratio of 2.5 ($Q_{\text{del}}$RC-AF660, red). (B) MALDI-TOF spectra of RC (purple) and AF660 conjugated RC (red). The RC spectrum shows three peaks corresponding to H, L and M subunits, whereas the RC-AF spectrum has two and one extra peaks for L and M, respectively, signifying selective and site-specific conjugation of the dye to the RC. (C) Fluorescence emission spectra of AF660 conjugated to BSA (blue) and quinone-depleted RC (red) with excitation at 600 nm. The spectra were corrected by detector response file and scaled by the dye absorbance at 600 nm. A 70% fluorescence intensity decrease is observed likely due to energy transfer from AF660 to quinone-depleted RC. (D) Fluorescence lifetime decay traces of AF660 conjugated to BSA (blue) and quinone-depleted RC (red) monitored at 698 nm ($\lambda_{\text{ex}} = 600$ nm).
The absorption spectrum of the dye-conjugated RC shows strong absorbance between 550 nm and 750 nm, where the RC itself absorbs only weakly (Figure 3.2A, S3.2-S3.5). There is a 4 nm shift in the absorbance and a 7 nm shift in the fluorescence of the AF660 dye when attached to the RC, compared to the free dye in solution, similar to that of the BSA-AF660 conjugate. The same trends were also observed for the RC-AF647 and RC-AF750 conjugates. For this reason, the BSA-dye conjugates were used as reference samples, rather than the free dyes in solution, for all spectroscopic measurements.

For some experiments, both quinones (QA and QB) were removed from the RC, so that the charge separated state (P+H+) recombines to the ground state in nanoseconds, to ensure a complete recovery of P before each laser excitation in transient absorbance measurements (see supporting information for details).12 AF660 conjugated to the quinone-depleted RC had an average dye-to-RC ratio of 2.5 and exhibited essentially the same spectroscopic properties as the quinone-containing RCs.

When the quinone-depleted RC-AF660 conjugate was excited at 600 nm, the steady state fluorescence from the AF660 dye was much reduced compared to that of BSA-AF660 conjugate with the same dye absorbance (~70% fluorescence quenching), as would be expected if there were substantial energy transfer from the dye to the RC pigments. The corresponding quenching of the fluorescence emission was found to be 59% and 60% for the RC-AF647 and RC-AF750 conjugates, respectively, compared to the BSA-conjugated controls. Although the fluorescence emission spectrum of AF750 overlaps better with the RC absorption between 700 nm to 900 nm than does the spectrum of AF660, the RC-AF660 conjugate has higher energy transfer efficiency.
Time-resolved fluorescence decay measurements of the various free dyes, BSA-dye conjugates and RC-dye conjugates were performed using time-correlated single-photon counting techniques. Kinetic analysis of all free dyes in solution revealed a biexponential decay (Table 3.1, Figure S3.6). The amplitude-weighted average lifetimes of each dye are 1.04 ns for AF647, 1.11 ns for AF660 and 0.56 ns for AF750. In contrast, exponential fitting of the fluorescence decay kinetics for each of the protein-dye molecule conjugates required three exponential components (Table 3.1). The average lifetimes for the BSA-conjugated dyes were comparable to or slightly longer than the free dyes in solution (0.99, 1.33 and 0.77 ns for BSA-AF 647, -AF660 and -AF750, respectively). The increase in the complexity of the fluorescence decay of the dye conjugated on the protein surface over the free dye is not surprising. As can be seen from the quantum yields of fluorescence (Table 3.1), the decay lifetime is dominated by nonradiative pathways (vibrational coupling between the ground and excited states). The vibrational manifold of the protein environment is more complex than that of a homogeneous solvent. The shift in the environment in the BSA-conjugates compared to the free dyes also gives rise to the small red-shift in the peak absorbance and emission spectra of the dyes upon conjugation (Figures 3.2A, S3.2-S3.5), presumably due to an overall change in polarity or polarizability of the environment.

A substantial decrease in the average fluorescence decay lifetime of each dye is observed in the RC-dye conjugates; lifetimes of 0.45 ns, 0.45 ns, and 0.37 ns, were measured for the RC-AF647 conjugate, the quinone-depleted RC-AF660 conjugate and the RC-AF750 conjugate, respectively. For the RC-AF647 and RC-AF660 conjugates, the decrease in the average lifetime is primarily due to an increase in the amplitude of the
Table 3.1. Fitting parameters for lifetime data.

<table>
<thead>
<tr>
<th>Sample</th>
<th>τ1 ns (amplitude %)</th>
<th>τ2 ns (amplitude %)</th>
<th>τ3 ns (amplitude %)</th>
<th>Average Lifetime (ns)*</th>
<th>Quantum Yield*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF647</td>
<td>Free</td>
<td>0.41 (6.9)</td>
<td>1.09 (93.1)</td>
<td>-</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>λex = 600 nm</td>
<td>BSA</td>
<td>0.05 (30.8)</td>
<td>0.64 (19.8)</td>
<td>1.73 (49.4)</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>0.07 (62.9)</td>
<td>0.46 (16.2)</td>
<td>1.61 (20.9)</td>
<td>0.45</td>
</tr>
<tr>
<td>AF660</td>
<td>Free</td>
<td>0.55 (19)</td>
<td>1.24 (81)</td>
<td>-</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>λex = 600 nm</td>
<td>BSA</td>
<td>0.10 (13.2)</td>
<td>0.81 (25.5)</td>
<td>1.82 (61.3)</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>0.04 (67.9)</td>
<td>0.31 (16.1)</td>
<td>1.46 (16.0)</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>QRC</td>
<td>0.05 (60.3)</td>
<td>0.47 (18.9)</td>
<td>1.60 (20.8)</td>
<td>0.45</td>
</tr>
<tr>
<td>AF750</td>
<td>Free</td>
<td>0.55 (98.6)</td>
<td>1.17 (1.4)</td>
<td>-</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>λex = 710 nm</td>
<td>BSA</td>
<td>0.12 (11.7)</td>
<td>0.73 (76.0)</td>
<td>1.63 (12.3)</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>0.06 (40.0)</td>
<td>0.38 (34.6)</td>
<td>0.85 (25.4)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

# Average lifetime is calculated as $\tau_{ave} = \Sigma_i A_i \tau_i / \Sigma_i A_i$, where $A_i$ and $\tau_i$ are the amplitude and the lifetime components.

*Quantum yield of fluorescence is calculated as $\Phi = \Phi_R \frac{I_A \eta^2}{I_R \eta_R^2}$, where $\Phi$ and $\Phi_R$ are the quantum yield of the sample and the reference, $A$ and $A_R$ are the absorbance of sample and reference at the excitation wavelength, $\eta$ and $\eta_R$ are the refractive indexes of solvents for sample and reference, respectively.

shortest (~50-ps) decay component (Tables 3.1 and S3.2). For AF750, the lifetimes of all three components in the decay decreased. The overall decrease in average fluorescence lifetime (dye excited state lifetime) is consistent with a substantial level of energy transfer from the dye to the reaction center cofactors. Again, the complexity of the decay in the reaction center conjugates is likely due to the heterogeneity of the local environment (static or dynamic) and its effects on both energy transfer (which is sensitive to transition dipole orientation of the donor and acceptor) and nonradiative decay via vibrational coupling to the ground state.

A comparison of the average lifetimes for the dye conjugated to the RC vs. the dye conjugated to BSA resulted in estimated energy transfer quantum yields of 55%, 66% and 52%, for RC-AF647, quinone-depleted RC-AF660 and RC-AF750, respectively, which is
in reasonable agreement with the results obtained from steady-state fluorescence (59%, 70% and 60%, respectively, see also table S3.4). Thus, in either time-resolved or steady-state fluorescence measurements, higher energy transfer efficiency is observed for the RC-AF660 conjugates compared to the RC-AF750 conjugates, even though the fluorescence spectrum of AF750 shows a larger overlap with the absorbance spectrum of the RC pigments than does the fluorescence spectrum of AF660. In the time domain, one can directly see that the AF660 dye has a longer intrinsic excited state lifetime than does the AF750 dye, corresponding to its high fluorescence quantum yield (Table 3.1). Thus there is more opportunity for the excited state of AF660 to transfer energy to P. This exemplifies the key role of donor excited state lifetime in the design of light harvesting systems.

**Figure 3.3.** (A) Light-minus-dark absorbance spectra of RC-AF660 (blue) and unconjugated RCs (red). A 2.2 fold enhancement in P⁺ formation is observed due to the enhanced absorption cross-section at 650 nm. (B) RC-AF647 shows a 2.8 fold enhancement in P⁺ formation over unconjugated RCs.

One would expect that energy transfer from a dye to RC pigments would lead to charge separation. Because charge separation in the RC occurs with near unity yield, the
amount of charge separation that takes place should track the energy transfer efficiency. The relative amount of RC charge separation was determined by comparing the light-minus-dark difference spectrum (the degree of ground state bleaching of the 865 nm P band) of the RC-AF660 conjugate with that of unconjugated RCs. Each sample was illuminated through a band-pass filter centered at 650 nm (bandwidth 10 nm). The light intensities used were low enough so that all signals increased linearly with intensity, ensuring that the amplitude of the P-band bleaching measured at 865 nm reflects the relative amount of the P⁺Qₐ⁻ formed in each sample. As shown in Figure 3.3, P⁺Qₐ⁻ in the unconjugated RCs shows an absorbance difference spectrum that involves negative and positive signals at 865 and 770 nm, respectively. The RC-AF660 conjugates show essentially the same absorbance change but 2.2-fold greater in magnitude than that of the unconjugated RCs, showing that the energy of the 650 nm photons absorbed by AF660 and transferred to the RC cofactors is effective in charge separated state formation (most of the 650 nm light absorbed by the RC-AF660 conjugates is absorbed by the dye, as shown in Figure 3.1B). Similarly, the AF647 conjugated RCs show a 2.8 fold enhancement in P⁺ formation over unconjugated RCs excited at 650 nm. The increase in enhancement corresponds to the higher absorption cross section at 650 nm of RC-AF647 compared to that of RC-AF660 (See Figure S3.8 for absorption data).
Figure 3.4. (A) Time-resolved transient absorption difference spectra of quinone-depleted RC-AF660 conjugates in the 530–730 nm region and (C) AF660 dye itself in solution. (B) Time-resolved transient absorption difference spectra of quinone-depleted RC-AF660 conjugates and (D) unconjugated quinone-depleted RCs in the 800-940 nm region (near the maximal ground state absorbance of the RC cofactor P). For all samples, $\lambda_{ex} = 650$ nm.

All of the processes, from excitation of the dye to charge separation in the RC, can be observed kinetically via transient absorbance spectroscopy. Absorbance difference spectra as a function of time for both the AF660 dye (650 – 750 nm) and the initial electron donor P, in the RC (800 – 940 nm with the peak at 860 nm) were monitored over a broad wavelength region at different time delays following excitation at 650 nm. At this
excitation wavelength, the AF660 dye contributes more than 95% of the sample absorbance.

The transient absorbance difference spectra induced by the bleaching of AF660 and P in the quinone-depleted RC-AF660 conjugates, recorded at various delay times, were compared with those of both the free dye and of unconjugated, quinone-depleted RCs (Figure 3.4). For the quinone-depleted RC-AF660 conjugates, the ground state bleaching signal in the 650–750 nm region appeared instantaneously and then recovered by about 80% during the first 200 ps (Figure 3.4A), indicating the disappearance of the excited state population of AF660. As the AF660 ground state absorbance recovered, an absorbance decrease associated with bleaching of the ground state spectrum of P developed in the 800–940 nm region (Figure 3.4B). In quinone-depleted RCs, the terminal charge separated state is $P^+H^-\Lambda^-$, which forms within a few ps $^{9a,13-15}$ but then lives for 10 – 20 nanoseconds.$^{9a,16}$ Thus, once formed, the bleaching of the ground state absorbance from P remains constant on the time scale of this measurement.

In contrast to the situation in quinone-depleted RC-AF660 conjugates, the ground state bleaching that is generated upon excitation of the unconjugated AF660 dye itself does not decay by very much during the initial 200 ps (Figure 3.4C). Instead, the dye bleaching decreases over a roughly 1 ns time period, consistent with its inherent excited state lifetime (Table 3.1). Similar results have been observed for the BSA-AF660 construct (Figure S3.9A). As a control, transient absorption spectra of quinone-depleted RCs without dye were recorded at two different delay times, when the RCs are dominated by the state $P^*$ (1 ps) and the $P^+H^-\Lambda^-\Lambda$ (1 ns), again excited at 650 nm (Figure 3.4D). Although the absorbance at 650 nm is weak in unconjugated RCs, some RCs are excited. As expected, the spectrum
at 1 ns has the same profile as that obtained from the dye-conjugated RC, consistent with formation of the long-lived charge separated state $P^+H_A^-$ in both samples. However, the spectrum of unconjugated, quinone-depleted RCs at 1 ps shows spectral changes expected for the direct excitation of the RC, forming the excited state of $P$ ($P^*$) rather than the absorbance changes associated with the excited state of the dye, as seen in the dye conjugated RCs. The $P^*$ signal consists of a large absorbance extending between the 860 nm region (ground state bleaching) and the 900 nm region (stimulated emission from $P^*$). The lack of stimulated emission signal in the quinone-depleted RC-AF660 conjugates (Figure 3.4B) is due to the low steady-state population of $P^*$; energy transfer forming $P^*$ from AF660 takes place in tens of ps, whereas the conversion of $P^*$ to $P^+$ takes only 3 ps.  

Figure 3.5. Transient absorbance kinetics (A) at 700 nm for unconjugated AF660 dye in solution (AF660, blue) and quinone-depleted RC-AF660 conjugates (Q$_{del}$RC-AF660, red); (B) Ground state bleaching of $P$ at 870 nm in unconjugated RCs (Q$_{del}$RC, blue) and quinone-depleted RC-AF660 conjugates (Q$_{del}$RC-AF660, red). Excitation is at 650 nm.
The ground state bleaching of the dye absorbance following a pulse directly exciting the dye (at 650 nm) should recover as the dye excited state decays. The dye ground state recovery kinetics was compared for the unconjugated AF660 dye and the quinone-depleted RC-AF660 conjugates (Figure 3.5A), probing at 700 nm. In agreement with the single photon counting measurements (Figure 3.2D) and the conclusions drawn from analysis of the time resolved spectra in Figure 4, the excited state lifetime of the AF660 in quinone-depleted RC-AF660 conjugates is much shorter than that of the free dye in solution, again supporting the conclusion that energy transfer is the dominant pathway of dye excited state decay in the RCs conjugated to dye. The absorbance change kinetics were fit with three exponential components. The fastest lifetime resulting from the fit was 25 ps. This is shorter than the ~50 ps lifetime obtained from multi-exponential fits of the single photon counting data, likely due to the higher time resolution of the transient absorbance measurements (0.1 ps vs. 40 ps). An average lifetime of 570 ps (25 ps (35%), 164 ps (29%) and 1418 ps (36%)) over the whole decay was determined for quinone-depleted RC-AF660 conjugates from transient absorbance measurements while AF660 in solution gave an average lifetime of 1130 ps (519 ps (30%) and 1390 ps (70%)) (Table S3.3). For comparison, the average lifetime of the BSA-AF660 conjugate used as a control was 1570 ps (531 ps (18%) and 1800 ps (82%)). A comparison of the average lifetimes for the dye conjugated to the RC vs. the dye conjugated to BSA resulted in an estimate of 64% for the overall energy transfer efficiency (Table S3.4), which is in reasonable agreement with the results obtained from steady-state fluorescence (70%) and time correlated single photon counting measurements (66%).
The relative amounts of P\(^+\) formed in RCs with and without AF660 conjugated were determined by comparing the extent of ground state bleaching at 870 nm (Figure 3.5B). Using excitation at 650 nm, where the unconjugated RC absorbs weakly, roughly a 2.7-fold increase in P\(^+\) formation was observed in the quinone-depleted RC-AF660 conjugate compared to the unconjugated, quinone-depleted RC at the same concentration, in agreement with the results of steady-state P\(^+\)Q\(_A\) formation monitored via light-dark difference spectroscopy (Figure 3.3).

If energy transferred from the AF660 dye to P results in P\(^+\) formation, one would expect that the kinetics of AF660 ground state recovery (700 nm) would match that of the formation of ground state bleaching due to formation of P\(^+\) (870 nm). As shown in Figure S3.7, this is indeed the case; the normalized traces show a fast decay of the dye and a concomitant formation of the P\(^+\) signal. The kinetic trace from unconjugated RC is shown for comparison and exhibits instantaneous formation of the P bleaching signal due to the direct excitation of RC cofactors.
**Figure 3.6.** Cytochrome c oxidation was monitored at 550 nm after 800 nm (A) and 650 nm (B) excitation. At 800 nm excitation, each of the samples shows similar kinetics, whereas using 650 nm excitation, the dye conjugated samples display much faster rates of cytochrome c oxidation compared to the wild type (RC-wild type) and cysteine mutated (RC) RCs. RC-AF647 has a faster rate than that of RC-AF660 due to its higher absorption cross-section at 650 nm. The absorbance change scales are different because the light intensities at 650 nm and 800 nm are not the same.

A key aspect of natural RC function is the ability to accept electrons from soluble cytochrome c. Given the proximity of the conjugated dye molecules to the cytochrome binding site, one might be think that dye would interfere with cytochrome binding and thus electron transfer to P+.

To explore this possibility further, cytochrome c was added to a solution of dye-conjugated RCs and the absorbance changes associated with cytochrome c oxidation were monitored at 550 nm. The RC-AF647 and RC-AF660 conjugate were able to oxidize soluble cytochrome c much more rapidly than unconjugated RCs when 650 nm excitation was used (Figure 3.6). In fact, the increase in the rate of cytochrome c oxidation observed using 650 nm excitation was at least as large as the increase in P+Q− formation seen in the light-minus-dark measurements (Figure 3.3), consistent with an increase in absorbance cross section at 650 nm and implying that the presence of the dye molecules did not substantially inhibit electron transfer to the cytochrome. As expected, dye conjugated and unconjugated samples showed a similar rate of cytochrome c oxidation using 800 nm excitation where all the samples have same absorption cross section.
3.5. Conclusion

Conjugation of any of the three dyes tested at positions near P in the RC results in a substantial increase in the effective absorbance cross section for charge separation in the visible part of the spectrum where the dyes absorb strongly, but the RC has weak absorbance. Further, it was possible to specifically place the dye molecules at positions that were well within the Förster energy transfer distance to P and yet did not substantially perturb the ability of P to either donate electrons to subsequence cofactors in the normal electron transfer sequence or to accept electrons from soluble cytochrome c.

The ability to functionally couple any of several different dyes to the RC makes it possible to tune the action spectrum of the system over a broad range. This type of model system also makes it possible to start defining the parameters involved in the design and construction of more complex molecular photonic devices, such as the effects of geometry, dye environment, dye excited state lifetime, and the type and conformational flexibility of dye conjugation chemistry.

3.6. References


Chapter 4

A DNA-Directed Light-Harvesting/Reaction Center System


4.1. Abstract

A structurally well-defined and spectrally tunable artificial light-harvesting system has been constructed in which multiple organic dyes attached to a 3-arm DNA nanostructure serve as an antenna conjugated to a photosynthetic reaction center isolated from *Rhodobacter sphaeroides* 2.4.1 (PDB 2J8C). The light energy absorbed by the dye molecules is transferred to the reaction center where charge separation takes place. The number of DNA 3-arm junctions per reaction center was tuned from 1 to 3. This DNA-templated multi-chromophore system serves as a modular light-harvesting antenna that is capable of being optimized for its spectral properties, energy transfer efficiency and photostability, allowing one to adjust both the size and spectrum of the resulting structures. This may serve as a useful test-bed for developing nanostructured photonic systems.

4.2. Introduction

During photosynthesis, light energy is collected by a large light-harvesting network and efficiently transferred to a reaction center (RC), which converts it to chemical energy via charge separation.\(^1\) The quantum efficiency of the charge separation reaction by the photosynthetic reaction center is nearly unity.\(^1d\) The architecture and spectral properties of
the light-harvesting system that surrounds the reaction center have evolved to meet the constraints of a broad range of different light conditions and environments. A number of researchers have attempted to mimic the natural photosynthetic apparatus by designing artificial light harvesting antenna systems\textsuperscript{2-5} for a variety of photonic applications.\textsuperscript{6}

To facilitate nanoscale photonic applications more broadly, the construction of artificial antenna systems that provide controllable light absorption, efficient energy transfer and improved photo-stability are desirable. Self-assembling proteins\textsuperscript{3} and dendrimers\textsuperscript{4} have been explored to create artificial antenna systems, but they lack a well-defined multi-chromophore geometry and stoichiometry. Synthetic porphyrin structures\textsuperscript{5} have been investigated to create artificial antennas connected to electron transfer complexes, but these generally have an absorption cross-section that is spectrally relatively narrow. DNA nanotechnology can be used to generate programmable, self-assembled nanostructures\textsuperscript{7} with multiple fluorophores at well-defined positions, and this approach has been used to create artificial light harvesting antenna systems. Double helical DNA structures, three-way junctions, seven helix bundles and several other DNA based antenna systems\textsuperscript{8} have been used to create artificial antennas with unidirectional energy transfer along an excited state energy gradient between chromophores that mimics the stepwise energy transfer in some of the natural photosynthetic systems. However, thus far these assemblies have lacked the ability to convert the light energy to redox energy via charge separation.

Recently, we have studied different dye molecules directly conjugated to reaction centers and explored the effects of altering the dye spectral and excited state properties on the efficiency of energy transfer and charge-separation.\textsuperscript{9} In this report we go a step further
and use a three-arm DNA nanostructure to organize multiple dye molecules and specifically assemble these nanostructured complexes with reaction centers, resulting in a geometrically programmable model system mimicking a natural photosynthetic apparatus.

**Figure 4.1.** (A) Modified structure of the reaction center (RC) from the purple bacterium, *Rhodobacter sphaeroides* 2.4.1 (PDB 2J8C) with sequences of the 3arm-DNA construct shown. The cofactors of the RC are colored and those active in electron transfer reactions involved in this report are designated by letters: P – bacteriochlorophyll pair, Bₐ – bacteriochlorophyll monomer, Hₐ – bacteriopheophytin, Qₐ – ubiquinone. The arrows point in direction of the 3’ end of the DNA strands. The 3’-Amine modified Strand-1 (purple) of the 3arm-DNA is conjugated to one of the Cys residues (shown in red) on the surface of the RC via a SPDP (N-succinimidyl 3-(2-pyridyldithio) propionate) linker. The other two strands (Strand-2 and -3 in green and red, respectively) are allowed to hybridize to Strand-1 to form the 3arm-DNA junction. Inter-Cys distances on the RC are marked as
dotted lines. The two stars on 3arm represent the positions of the two dye molecules, where the cyan star corresponds to either Cy3 or AF660, and the pink star corresponds to either Cy5 or AF750. (B) is a representative absorption spectra of RCs that have on average 2.3 of the 3arm-Cy3-Cy5-RC(3DNA) nanostructures attached (referred to as 3CC) and (C) is an absorbance spectrum of RCs that have an average of 2.1 of the 3arm-660-750-RC(3DNA) nanostructures attached (referred to as 3-6-7). The absorbance spectra of panels B and C show enhanced absorbance cross-section in the spectral regions 500-700 nm or 550-800 nm, respectively, where the RC absorbance is relatively low.

4.3. Materials and Methods

4.3.1. Reaction Center Protein Preparation: Among total eight mutations in the RC, five of them serve to replace the 5 wild type cysteines with serine and alanine, and the remaining three mutations are for introducing cysteines at the P side of the RC, by replacing wild type amino acids (glutamic acid or asparagine) with Cys at the point of interest (Figure S1). The mutations are as follows: (H)C156A, (H)C234S, (M)E100C, (L)C92S, (L)C108S, (L)C247S, (L)E72C and (L)N274C. Furthermore, the engineered RC contains a six-histidine tag at the C-terminus of the H subunit, to facilitate purification with a Ni-sepharose affinity column.

RCs were isolated from a mutant derived from R. sphaeroides 2.4.1. 2 L of modified LB medium, containing 810 μM MgSO4, 510 μM CaCl2 and 4 mM NaCl, was used to grow cells at 30°C for 3.5 days. The cells were pelleted and resuspended in 50 mM phosphate buffer (pH 8) containing 150 mM NaCl. The cells were then lysed by passing through a French press, followed by addition of small amount of DNase. After removal of any unbroken cells and large cell debris via centrifugation (9000 g for 10 minutes), the
remaining supernatant was treated with imidazole (final concentration 5 mM) and the RC protein was solubilized by adding N,N-Dimethyldodecylamine N-oxide (LDAO, final concentration 0.4% by volume). After 20 min incubation at 22°C, the solution was centrifuged at 14000g followed by Ni-sepharose column purification. The eluted RC was dialyzed overnight at 4°C against dialysis buffer using 50 kD molecular weight cutoff membrane (Amicon), to remove imidazole and excess LDAO. The concentration of the purified RC was measured using absorbance at 804 nm (ε ~288000 M⁻¹cm⁻¹). Below is the list of the buffers used with their respective composition.

- Modified LB medium: 2L medium contains 20 gram tryptone, 10 gram yeast extract, 480 mg NaCl, 1020 µL CaCl₂ (1M stock), 1620 µL MgCl₂ (1M stock).
- Resuspension buffer: 50 mM phosphate buffer, 150 mM NaCl, pH 8.
- Wash buffer: 50 mM phosphate buffer, 0.1% LDAO, 150 mM NaCl, 5 mM imidazole, pH 8.
- Elution buffer: 50 mM phosphate buffer, 0.1% LDAO, 150 mM NaCl, 100 mM imidazole, pH 8.
- Column wash buffer: 50 mM phosphate buffer, 0.1% LDAO, 300 mM NaCl, 250 mM imidazole, pH 8.
- Dialysis buffer: 15 mM Tris, 0.025% LDAO, 150 mM NaCl, 1 mM EDTA, pH 8.

4.3.2. RC-DNA Conjugation and Purification: An amine-modified DNA (Strand 1, 5’-TCGCTAGGAACGG ATT TT-3’) of ~400 µM in 1×PBS, pH 7.6 was treated with 20 fold excess of 50 mM SPDP (N-succinimidyl 3-(2-pyridyldithio) propionate) in dimethyl sulfoxide (DMSO), followed by addition of 1M NaHCO₃ (~1/10 of total volume of DNA-SPDP mixture, to adjust pH) and the mixture was shaken gently
for 3 hours at room temperature. The DNA-SPDP conjugate was purified with Nap-10 desalting column (GE Healthcare) and then washed 3 times with 1×PBS using 3kD molecular weight cut-off filter (Amicon) to remove the excess SPDP. The RC was treated with 8 fold excess of 50 mM TCEP-HCl (Tris(2-carboxyethyl)phosphine hydrochloride) for 30 min at 4°C, followed by washing with 1×PBS, 0.025% LDAO, pH 8 using 50kD molecular weight cut-off filter (Amicon) to remove excess TCEP-HCl.

A 10 fold excess of DNA-SPDP conjugate was mixed with TCEP-HCl treated RC and left for ~6 hours at 4°C in gentle mixing condition. Then the mixture was treated with 10 mM phosphate buffer with high salt (1.5 M NaCl, 0.025% LDAO, pH 8), followed by washing 3 times with 10 mM phosphate, 0.025% LDAO, pH 8 buffer to get rid of any NaCl.

**Scheme 4.1.** RC-DNA conjugation using SPDP as bi-specific cross-linker.

The sample was then run through anion exchange column (Mono Q 4.6/100 PE, product code-17-5179-01) using fast protein liquid chromatography (FPLC) system (AKTA purifier). The desired fractions containing RC-DNA conjugates with different
protein:DNA ratios were washed with dialysis buffer as mentioned earlier. Composition of buffer used for anion exchange column: Equilibration buffer: 10 mM phosphate, 0.025% LDAO, pH 8; Elution buffer: 10 mM phosphate, 1M NaCl, 0.025% LDAO, pH 8.

4.3.3. DNA-dye Conjugation and Purification: Cy3 and Cy5 labeled strands (HPLC purified) (5’-CGCTACATCA/iCy3/TCCTAGCGA-3’ and 5’-/5Cy5/ATCCGTTGATGTAGCG-3’) were purchased from IDTDNA and used as received. Alexa Fluor dye (AF660 and AF750) labeled DNA strands were prepared in the lab as following.

Amine modified DNAs for dye conjugation were synthesized on a DNA synthesizer (ABI 394 DNA/RNA Synthesizer, Applied Biosystems) via standard protocols by using CPGs (1 μmole scale) with a coupling time of 5 min for amine modified phosphoramidite (amino-modifier C6 dT phosphoramidite for Strand 3 and 5’-amino-modifier C6 phosphoramidite for Strand 2; both purchased from Glen Research). The oligonucleotide was cleaved from the resin by treatment with 1:1 volume mixture of NH₄OH (28% in water) and methylamine (40% in water) for 2 hours at 50°C, and then purified using HPLC (Agilent Technologies 1200 series) with a Phenomenex-C18 column (Solvent A: 100 mM triethylammonium acetate, pH 7; Solvent B: acetonitrile; Flow rate: 4 mL/min). The fractions containing the desired oligonucleotides were collected and lyophilized. After redissolved in water, they were precipitated in 70% cold ethanol. The pellet of oligonucleotide was washed with 70% ethanol and dried under vacuum, followed by dissolving in 0.1 M sodium tetraborate buffer (Na₂B₄O₇.10H₂O, pH 8.5) to get a final concentration of ~200 μM.
A 10-fold excess of Alexa Fluor dye (Invitrogen, amine reactive Alexa Fluor 660 and -750) from a stock solution of ~ 15 mM concentration (dissolved in DMSO) was added to the above DNA solution and incubated overnight with gentle shaking at room temperature. DNA was precipitated using 3 M NaCl and ethanol, and pelleted. The pellet was dissolved in water followed by HPLC purification (same as described above). The fraction containing the Dye-DNA conjugate was collected and lyophilized.

**Scheme 4.2. DNA-Alexa Fluor dye conjugation.**

MALDI-mass spectrometry (Applied Biosystem Voyager System 4320 and Bruker Microflex) analyses were carried out before and after the dye conjugation, using 3-hydroxypicolinic acid as the matrix (Figure S4.17).

**4.3.4. 3arm-RC Preparation:** Free 3arm-DNA constructs were prepared by mixing stoichiometric quantities of three DNA strands in TAE/Mg\(^{2+}\) buffer and subsequent annealing from 90°C to 10°C. After annealing the structures were purified by 8% nondenaturing PAGE and transferred in Tris buffer (15 mM Tris, 20 mM Mg\(^{2+}\), 150 mM NaCl, 1 mM EDTA, pH 8). The stoichiometric formation of the 3arm-DNA constructs are confirmed by native PAGE image (shown in Figure S4.2)

First strand-2 and -3 were annealed in the above mentioned Tris buffer from 90°C to 10°C and then mixed with DNA conjugated RC (strand-1 conjugated with RC), with 1.5 fold molar excess followed by annealing from 30°C to 10°C over 12 hrs. The mixture was
then purified with 50kD molecular weight cut-off filter (Amicon) using above mentioned Tris buffer containing 0.025% LDAO, to remove the excess DNA strands.

**4.3.5. Absorption and Fluorescence Spectroscopy:** Absorption spectra were measured using a quartz cell with 1 cm path length in a Jasco V-670 spectrophotometer. Steady state fluorescence spectra were measured in a Nanolog Fluorometer (Horiba Jobin Yvon), with a quartz cuvette of 1 cm path length. All the steady state emission spectra were corrected for the wavelength dependence of the response of the detection system.

**4.3.6. Time-Correlated Single-Photon Counting Measurements:** Fluorescence lifetime measurements were analyzed by time-correlated single-photon counting. For that we have used a fiber supercontinuum laser (Fianium SC450) as the excitation source, which was operated at 20 MHz. The laser output was sent through an Acousto-Optical Tunable Filer (Fianium AOTF) to obtain excitation pulses at wavelengths of 510 nm, 600 nm, 620 nm and 740 nm. Fluorescence emission was collected at a 90° geometry setting and detected using a double-grating monochromator (Jobin-Yvon, Gemini-180) and a microchannel plate photomultiplier tube (Hamamatsu R3809U-50). The polarization of the emission was 54.7° relative to that of excitation. Data acquisition was done using a single photon counting card (Becker-Hickl, SPC-830). The typical instrument response function had a full width half maximum of 50 ps, measured from the light scattered from sample at the excitation wavelength. The data were fitted using a locally written software package ASUFIT.

**4.3.7. Calculation of FRET efficiency, average lifetime of dye molecules and decay rate constants for different processes:** FRET efficiencies (E) were calculated according to the following equation:
\[ E = 1 - \frac{I_{DA}/A_{DA}}{I_D/A_D} \] (1)

Where \( I_{DA} \) and \( I_D \) are the integrated area of fluorescence from the donor with and without an acceptor. \( A_{DA} \) and \( A_D \) are the absorbance of the donor at excitation wavelength with and without an acceptor.

Average lifetime was calculated using the following equation.

\[ \tau_{ave} = \frac{\sum_i A_i \tau_i}{\sum_i A_i} \] (2)

Where \( A_i \) and \( \tau_i \) are the fitted amplitude and the lifetime components.

The energy transfer efficiency calculated from lifetime measurements were achieved using the following equation.

\[ E_{\text{lifetime}} = 1 - \frac{\tau_{ave,DA}}{\tau_{ave,D}} \] (3)

Where \( \tau_{ave,DA} \) and \( \tau_{ave,D} \) are the average lifetime of the donor with and without an acceptor obtained from the TCSPC data.

The measured average lifetime (\( \tau_1 \)) of Cy3 in 3arm-Cy3 is 1.79 ns (Table S4.1).

\[ \tau_1 = \frac{1}{k_{r,Cy3} + k_{nr,Cy3}} \] (4)

Where \( k_{r,Cy3} \) and \( k_{nr,Cy3} \) are the radiative and nonradiative decay rate constants of Cy3. So, \( k_{r,Cy3} + k_{nr,Cy3} = 0.55 \text{ ns}^{-1} \).

In the case of 3arm-Cy3-Cy5, the measured average lifetime of Cy3 (\( \tau_2 \)) is 0.50 ns (Table S4.1).

\[ \tau_2 = \frac{1}{k_{Cy3-Cy5} + k_{r,Cy3} + k_{nr,Cy3}} \] (5)
Where $k_{Cy3-Cy5}$ is the rate constant for Cy3 to Cy5 energy transfer, which is calculated to be 1.45 ns$^{-1}$ by combing (4) and (5).

Now, in the case of 1IC, the average lifetime of Cy3 ($\tau_3$) is 1.06 ns (Table S4.1).

$$\tau_3 = \frac{1}{k_{Cy3-RC} + k_{r,Cy3} + k_{nr,Cy3}} \quad (6)$$

Where $k_{Cy3-RC}$ is the rate constant for Cy3 to RC energy transfer, which is calculated to be 0.39 ns$^{-1}$ by combing (4) and (6).

Now, in the case of 1CC, the average lifetime of Cy3 ($\tau_4$) is

$$\tau_4 = \frac{1}{k_{Cy3-RC} + k_{Cy3-Cy5} + k_{r,Cy3} + k_{nr,Cy3}} \quad (7)$$

Using the above values, $\tau_4$ is calculated to be 0.42 ns, whereas the experimentally observed lifetime of Cy3 in 1CC is 0.28 ns (Table S4.1).

Similarly, the calculated values of $k_{AF660-RC}$, $k_{AF660-750}$, and $(k_{r,AF660} + k_{nr,AF660})$ are 0.20 ns$^{-1}$, 0.30 ns$^{-1}$, 0.59 ns$^{-1}$. So, the calculated lifetime of AF660 in 1-6-7 is 0.92 ns, whereas the experimental value is 0.90 ns.

The closeness of the numbers obtained from different measurements indicates that the experimental measurements are all consistent with each other, and the physical pictures of the step-wise energy transfers are accurate.

### 4.3.8. Cytochrome c Oxidation Experiment:

Before measuring cytochrome c oxidation kinetic, bovine heart cytochrome c was reduced by treating with 10-fold molar excess of sodium ascorbate in 10 mM sodium phosphate buffer (pH 6.9), followed by purification with Nap-25 column (GE Healthcare). The oxidation kinetics of cytochrome c in presence of 3arm-RC were measured by monitoring the change in the absorbance at 550
nm in the presence of a 650 nm or 800 nm excitation beam. The 800 nm and 650 nm excitation light beams were generated by passing white light (Dolan-Jenner MH-100 Metal Halide Fiber optic illuminator) through 800 nm band pass filter (FB800-40, FWHM 40 nm), and through RG610 (long pass) and IF650 (band pass, FWHM 10 nm), respectively. The sample contained 0.1 μM 3arm-RC, 100-fold molar excess of decylubiquinone (extinction coefficient at 409 nm in ethanol = 343 M⁻¹cm⁻¹) and 10-fold molar excess of reduced cytochrome c in above mentioned dialysis buffer (see section 4.3.1).

4.3.9. **Light-Minus-Dark Experiment:** The light-minus-dark experiments were performed by measuring the absorbance spectra of sample taken in the dark (dark spectra) and in presence of 550 nm (bandwidth ~10 nm) continuous light, and finally subtracting the dark spectra from the spectra obtained at 550 nm excitation (light spectra). The samples were illuminated with 550 nm light for 6 minutes prior to measurement. The path of the excitation light was perpendicular to the path of the probe light from the UV-Vis absorbance spectrophotometer. The excitation light at 550 nm was obtained using a white light source (Dolan-Jenner MH-100 Metal Halide Fiber optic illuminator) passed through two filters (BG 38 and IF550; 10 nm band pass, Figure S4.16). For all measurements, samples contained a 50-fold excess of 1,10-phenanthroline compared to the RC concentration.

4.3.10. **Time-resolved Ultrafast Transient Absorption Spectroscopy:** Narrowband pump-probe system was used to measure transient absorption kinetic. A 100 fs laser pulses at 800 nm were generated from a regenerative amplifier system (Tsunami and Spitfire, Spectra-Physics) operated at 1 kHz, part of which was used to generate the pump excitation pulses at 650 nm by using an optical parametric amplifier (IR OPA,
Spectra-Physics). Absorbance changes probed at a specific wavelength were recorded using a photodiode as a function of the delay time after the pump. The absorbance changes as a function of time were fit to a multiple exponential model using locally written software, ASUFIT (Figure S4.18).

4.4. Results and Discussion

Two different pairs of DNA-conjugated chromophores are used in this study: Cy3 and Cy5, or Alexa Fluor 660 and Alexa Fluor 750. Cy3 acts as the donor and Cy5 as the acceptor in the first pair, and AF660 acts as the donor and AF750 as the acceptor in the second pair. The fluorophores were chosen so that there is significant spectral overlap between emission of the dyes and the absorption of RC to facilitate efficient energy transfer, and so that there is a substantial increase in the absorption cross section in the spectral regions where the absorbance of RC alone is low. A very simple three-arm DNA structure was designed to assemble the two dye molecules in a geometrically defined manner and to avoid chemical modification of any DNA strands with more than one dye (to reduce cost and synthetic complexity). Two of the strands (Strand-2 and -3) in the three-arm DNA contain the dye molecules, and the other one (Strand-1) is conjugated to the RC through a covalent cross-link.

The three dimensional structure of the RC complex from *Rhodobacter sphaeroides* 2.4.1 (PDB 2J8C) is depicted in Figure 4.1A, and consists of three subunits H, M and L. There is a total of ten cofactors associated with the L/M transmembrane region of the structure, including a dimer of bacteriochlorophylls (P), two bacteriochlorophylls (B_A and B_B), two bacteriopheophytins (H_A and H_B), two ubiquinone-10 (Q_A and Q_B), one carotenoid and one iron ion (Fe^{2+}). The special pair P is the primary donor of electrons in
the light-driven electron transfer process, which subsequently transfers electron to QA via 
BA and HA, forming a long-lived charge-separated state P⁺QA⁻. With ubiquinone located at 
the QB site, electron transfer occurs from QA⁻ to QB forming P⁺QB⁻.¹¹

A genetically modified RC was used in these studies and contained a total of eight 
mutations, five of them to replace the five wild-type cysteines with serine or alanine, and 
the remaining three to replace three selected wild-type amino acids (asparagine or glutamic 
acid) with cysteine residues at specific locations on the surface of the RC that are close to 
the primary electron donor, P.⁹b,¹² Two of the new Cys residues are located on the surface 
of the L subunit (L72, L274) and the other one is on the surface of the M subunit (M100).

A 3'‑Amine modified Strand-1 was conjugated to these Cys residues of the RC by 
using a SPDP (N-succinimidyl 3-(2-pyridyldithio) propionate) cross-linker, and 
conjugated material was subsequently purified by fast protein liquid chromatography 
(FPLC) (Figure 4.2). The first three peaks on the FPLC trace represent conjugates with 
different DNA/protein ratios, i.e. one, two or three DNA strands conjugated to the RC, 
respectively. This assignment was based on the UV-VIS absorbance spectra of the fractions 
and the extinction coefficients of the DNA and the RC protein. The last peak in the 
chromatograph has no absorbance at 365 nm (the Soret absorbance band of the RC), 
indicating that it is likely excess free ssDNA. Dye-labeled Strand-2 and -3 are then allowed 
to hybridize to the purified Strand 1-conjugated-RC to create 3arm-RC conjugates with 
one, two or three 3-arm junctions on each RC.
Figure 4.2. FPLC purification trace of DNA (Strand-1) conjugated RCs. Chromatographs at 260 nm (green), 280 nm (red) and 365 nm (blue) are shown. The absorbance bands at 260 nm and 280 nm are from both RC and DNA, whereas the absorbance bands at 365 nm are from RC. The fractions from each of the peaks were collected separately and their respective absorbance spectra measured. The UV absorbance maxima for the first, second and third peaks in the chromatograph are at 271 nm, 268 nm and 266 nm, respectively, with a relative increase in the absorbance intensity (compared to the absorbance peak at 800 nm), which shows that there are different ratios of DNA conjugated to the RC (DNA:RC = 1:1, 2:1 and 3:1). The last peak in the chromatograph has no absorbance at 365 nm, indicating it is free ssDNA.
Cy3-modified Strand-3 and Cy5-modified Strand-2 were purchased from Integrated DNA Technologies (IDTDNA). AF660-modified Strand-3 and AF750-modified Strand-2 were synthesized by reacting amine-modified DNA (Strand-2 or -3, synthesized using a DNA synthesizer) with the succinimidyl ester of the corresponding dye (purchased from Invitrogen), which was subsequently purified by reverse phase HPLC and characterized using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectroscopy.

**Table 4.1. 3arm-to-RC ratio of different constructs**

<table>
<thead>
<tr>
<th>Dye</th>
<th>Sample</th>
<th>Abbreviation</th>
<th>3arm/RC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cy3/Cy5</td>
<td>3arm-Cy3-RC(1DNA)</td>
<td>1C</td>
<td>0.75 ±0.05</td>
</tr>
<tr>
<td></td>
<td>3arm-Cy3-RC(2DNA)</td>
<td>2C</td>
<td>1.65 ±0.05</td>
</tr>
<tr>
<td></td>
<td>3arm-Cy3-RC(3DNA)</td>
<td>3C</td>
<td>2.35 ±0.05</td>
</tr>
<tr>
<td></td>
<td>3arm-Cy3-Cy5-RC(1DNA)</td>
<td>1CC</td>
<td>0.8 ±0</td>
</tr>
<tr>
<td></td>
<td>3arm-Cy3-Cy5-RC(2DNA)</td>
<td>2CC</td>
<td>1.65 ±0.05</td>
</tr>
<tr>
<td></td>
<td>3arm-Cy3-Cy5-RC(3DNA)</td>
<td>3CC</td>
<td>2.2 ±0.1</td>
</tr>
<tr>
<td>AF660/AF750</td>
<td>3arm-660-RC(1DNA)</td>
<td>1-6</td>
<td>0.85 ±0.15</td>
</tr>
<tr>
<td></td>
<td>3arm-660-RC(2DNA)</td>
<td>2-6</td>
<td>1.6 ±0</td>
</tr>
<tr>
<td></td>
<td>3arm-660-RC(3DNA)</td>
<td>3-6</td>
<td>2.15 ±0.05</td>
</tr>
<tr>
<td></td>
<td>3arm-660-750-RC(1DNA)</td>
<td>1-6-7</td>
<td>0.9 ±0.1</td>
</tr>
<tr>
<td></td>
<td>3arm-660-750-RC(2DNA)</td>
<td>2-6-7</td>
<td>1.65 ±0.05</td>
</tr>
<tr>
<td></td>
<td>3arm-660-750-RC(3DNA)</td>
<td>3-6-7</td>
<td>2.0 ±0.1</td>
</tr>
</tbody>
</table>

The 1C, 2C or 3C (Abbreviations as in Table 4.1) constructs were created by assembling Strand-2 (unmodified) and Cy3-modified Strand-3 with the FPLC fraction that contained conjugates of one, two or three Strand-1 conjugates per RC, respectively. The spectra of these structures show enhanced absorbance between 500-580 nm relative to the RC alone, due to the addition of absorbance from Cy3 in this spectral region (Figure 4.3A and S4.5). A 3arm DNA nanostructure-to-RC ratio of 0.75 ±0.05, 1.65 ±0.05, and 2.35 ±0.05 were calculated based on the absorbance spectra for 1C, 2C and 3C. The observed yield of assembly for the fully loaded 3-arm DNA junction on the RC was 75-80%. This <100% yield may be due to local steric effects near the protein surface that reduce the DNA
hybridization yield. Assembled constructs with both Cy3 and Cy5 are named 1CC, 2CC and 3CC (Table 4.1), and show 3arm DNA nanostructure-to-RC ratios of 0.8 ±0, 1.65 ±0.05, and 2.2 ±0.1, respectively (Figure 4.3B and S4.6-4.8). Apparently adding the second dye molecules (covalently modified on the 5’ of Strand-2) did not affect the DNA hybridization yield. When both Cy3 and Cy5 are present, they absorb significantly between 500 and 680 nm. The antenna constructs made with both AF660 and AF750 provide strong absorbance between 600 and 800 nm (Figures 4.3C-D and S4.9-S4.12). The ratios of 3arm-to-RC for the different constructs are listed in Table 4.1.

Figure 4.3: Absorption spectra of the 3arm-dye-RC constructs. (A) 3arm-Cy3-RC(3DNA) or 3C, (B) 3arm-Cy3-Cy5-RC(3DNA) or 3CC, (C) 3arm-660-RC(3DNA) or 3-6 and (D)
3arm-660-750-RC(3DNA) or 3-6-7. The spectrum of RC alone (black trace) is included in each panel for comparison.

The FRET (Förster resonance energy transfer) process for each construct was investigated using both steady-state and time-resolved fluorescence spectroscopy techniques. The free 3arm-DNA constructs with respective dye(s) attached (without the RC) were used as reference samples for these experiments (Figure S4.3-S4.4). Upon exciting 1C at 510 nm, 42% of the Cy3 emission was quenched compared to that of 3arm-Cy3, presumably due to energy transfer from Cy3 to the RC. In the case of 1CC, there was an 83% decrease in Cy3 emission intensity compared to that of 3arm-Cy3. The greater decrease in fluorescence of Cy3 when Cy5 was present is attributed to the summation of multiple energy transfer pathways, which include a direct energy transfer from Cy3 to RC and a stepwise energy transfer from Cy3 to Cy5 to the RC. The same construct shows a 51% decrease in total fluorescence intensity integrated from 520 nm to 850 nm, compared with that of the 3arm-Cy3-Cy5 alone with no RC (Figure S4.6). On excitation of Cy5 at 620 nm, the direct FRET efficiency of Cy5 to the RC is 58%, using the emission of the 3arm-Cy3-Cy5 as a reference with the same excitation.

Similar experiments were performed on all the other 3arm-dye-RC constructs, and the energy transfer efficiency values are shown in Figure 4.4. Samples with different ratios of 3arm-dye conjugate to RC (such as 1C, 2C and 3C, or 1-6, 2-6 and 3-6) all yielded similar energy transfer efficiency values. This is due to the fact that although there are multiple dye molecules on the assembled structures, the probability of exciting more than one dye associated with a RC at a time is very low due to the continuous nature and low intensity of the excitation light. Moreover, as expected, the efficiency of energy transfer
from AF650 to the RC is higher than the efficiency of Cy3 transfer to the RC. This is presumably due to the greater spectral overlap between the emission of AF660 and the absorbance of the RC compared to Cy3. However, even though AF750 has a greater spectral overlap with RC than does Cy5, it has a lower energy transfer efficiency to RC than Cy5 does, and this results in a higher overall energy transfer efficiency of the Cy3-Cy5 pair to the RC than the AF660-AF750 pair. We have observed similar phenomena earlier,⁹b and the reason is the low intrinsic lifetime of AF750 compared to that of Cy5.

**Figure 4.4.** Energy transfer efficiency of 3arm-DNA conjugated RC calculated from steady-state data. (A) 1C, 2C, 3C, 1CC, 2CC and 3CC. (B) 1-6, 2-6, 3-6, 1-6-7, 2-6-7 and 3-6-7. (See Table 4.1 for a definition of the abbreviations.) The green bars show energy transfer efficiency calculated by comparing fluorescence from the RC containing complex with that from the 3arm-DNA consisting of only the initial energy transfer donor, which is either Cy3 or AF660. The blue and red bars are energy transfer values calculated using the 3arm-DNA consisting both the dyes (Cy3-Cy5 or AF660-AF750) without the RC attached as the fluorescence reference. The Blue bar results from direct excitation of the initial
energy transfer donor (Cy3 or AF660). The red bar results from direct excitation of the intermediate energy transfer dye (Cy5 or AF750).

Time-resolved fluorescence analysis was performed using time-correlated single-photon counting (TCSPC) (Figure 4.5, S4.13-4.14) excited by a pulsed laser. The decay traces of individual dye constructs (one dye on the 3-arm construct without the RC) could be fitted adequately with biexponential decay kinetics (Tables S4.1-S4.4). The amplitude-weighted average lifetimes that resulted were 1.79 ns for Cy3, 1.65 ns for Cy5, 1.68 ns for AF660, and 0.64 ns for AF750. In contrast, fitting the fluorescence decays for each of the 3arm-dye-RC constructs required three or four exponential components (Tables S4.1-S4.4). For example, considering the decay profiles of Cy3 in various samples ($\lambda_{ex} = 510$ nm and $\lambda_{em} = 565$ nm in Figure 4.5), a substantial increase in the fluorescence decay rate is seen with the RC conjugates (average lifetimes of 3C and 3CC are ~1.17 ns and ~0.25 ns, respectively). This follows the same trend as the steady-state energy transfer measurements and again implies that a significant amount of energy transfer takes place from the dye to the RC. Similar decay patterns were observed for the Alexa Fluor dye sets (Figure S4.14). Starting with the lifetime data for the dyes alone (without RCs) or one dye with the RC, the rate constants for the various component processes can be determined as described in the supplemental material. In particular, the rate constant for the decay of Cy3 in the absence of Cy5 or the RC is 0.55 ns$^{-1}$, the rate constant for energy transfer from Cy3 to the RC is 0.39 ns$^{-1}$, and the rate constant for energy transfer from Cy3 to Cy5 is calculated to be 1.45 ns$^{-1}$. If one uses the rate constants for these individual processes to predict the decay lifetime of Cy3 in the fully assembled complex (1CC), it is calculated to
Figure 4.5. Cy3 fluorescence decay profile of free 3arm-DNA and 3arm-DNA conjugated to RC with various ratios, with Cy3 alone (1C, 2C, 3C) on the constructs or with both Cy3 and Cy5 (1CC, 2 CC, 3CC) on the constructs, monitored at 565 nm ($\lambda_{ex} = 510$ nm).

be 0.42 ns, whereas the experimental average lifetime is 0.28 ns. Similarly for 1-6-7, the calculated decay lifetime of AF660 is 0.92 ns, whereas the experimentally observed lifetime is 0.90 ns. The approximate agreement of the decay times for the full nanostructures based on the kinetic constants estimated for individual component reactions indicates that the experimental measurements are internally consistent with each other, and consistent with an overall picture of step-wise energy transfer.

Further evidence of a stepwise energy transfer process is provided by the rise in the emission of Cy5 and AF750 in the TCSPC experiment, upon excitation of Cy3 and AF660, respectively, in the two-dye complexes (Figure S4.13-4.14). This is due to the energy transfer from the initial donor (Cy3 or AF660) to the intermediate dye (Cy5 or AF750),
which results in an initial increase in the excited-state population of the intermediate. A comparison of the average lifetimes of the dyes in the 3arm-RC constructs vs. that in the 3arm-only constructs result in estimated energy-transfer efficiencies from the dye to RC (Figure S4.15), which are in reasonable agreement with the results obtained from the steady-state fluorescence intensity measurements. Like the steady-state measurements, similar energy-transfer efficiencies are observed for samples with different numbers of DNA-dye constructs per RC. Again, in the case of time-resolved measurements, higher energy-transfer efficiency is observed for constructs that contain Cy5 compared to AF750, even though the fluorescence spectrum of AF750 overlaps better with the absorbance of the RC than does Cy5. This is because AF750 has a shorter excited state lifetime (0.64 ns) than does Cy5 (1.64 ns), giving the Cy5 excited state a greater probability of transferring energy to the RC before decaying by other pathways. Similar results were obtained previously when dye molecules with different lifetimes were conjugated directly to the RC.9b

Because charge separation in the RC has almost unity yield, the amount of charge separation that takes place correlates with the energy transfer efficiency.9b The relative amount of charge separation in the RC was investigated by measuring the light-minus-dark difference absorbance spectra of the different dye-DNA-RC complexes. The light-minus-dark difference spectra were obtained by substracting the absorbance spectrum of a sample taken in the dark from the absorbance spectrum taken under continuous illumination at 550 nm (Cy3 absorbance peak, 10 nm bandwidth). The light intensity at 550 nm was kept low enough to ensure the light-minus-dark signals changed linearly with the light energy absorbed. Under low light conditions, no RC is excited more than once during the ~100
Figure 4.6. Light-minus-dark difference absorbance spectra of RCs with and without conjugation to a 3arm DNA nanostructure-dye complex.

ms lifetime of $P^+Q_A^-$, avoiding artifacts due to photopumping. A 1.3 fold absorbance change at 862 nm (reflecting $P^+$ formation) was observed for 3C compared to the RC alone, implying enhanced charge separated state formation due to the increased absorbance cross section at 550 nm, confirming that photons absorbed by Cy3 result in energy transfer to RC cofactors (Figure 4.6). Similarly, 3CC shows a 1.8 fold enhancement in $P^+$ formation over unconjugated RCs. The enhanced $P^+$ formation in 3CC compared to 3C presumably results from the higher efficiency of energy transfer from Cy3 to Cy5 to RC compared to direct Cy3 to RC transfer (Figure 4.4). The insertion of Cy5 between Cy3 and the reaction center results in two relatively efficient transfer steps (better spectral overlap and shorter distance) compared to the single Cy3 to RC transfer. Like the energy transfer efficiency results obtained from both the steady state and the time resolved fluorescence
measurements, the relative intensity of P\(^+\) formation is similar for samples with different numbers of 3-arm DNA nanostructures conjugated to each RC (i.e., 1C, 2C and 3C vs. 1CC, 2CC and 3CC).

In the natural system, the RC operates in conjunction with the cytochrome \(bc_1\) complex, cytochrome \(c_2\), and a quinone pool, to convert light energy into a proton motive force.\(^{13}\) In this process, the oxidized initial electron donor of the RC, P\(^+\), that is formed upon light-driven electron transfer is subsequently reduced by cytochrome \(c_2\), which docks to the periplasmic face (P side) of the RC. In our artificial antenna system, the 3-arm-DNA structures are located on the P side of RC, and so one might expect that this conjugation of DNA close to the docking site of cytochrome would hinder cytochrome binding as well as the electron transfer process from cytochrome to P\(^+\). To explore this possibility, a 10-fold molar excess of reduced cytochrome \(c\)\(^{14}\) and a 100-fold molar excess of decylubiquinone were added into a solution of 3-arm DNA-dye-RC constructs, and the absorbance intensity change at 550 nm (an absorbance decrease at this wavelength reflects the oxidation of cytochrome \(c\)) was measured, while either exciting the RC directly or the dye directly.\(^9,15\) Using 800 nm excitation (direct excitation of the RC), where both the Cy3 and Cy5 have no absorbance, the wild type RC, the Cys-modified RC, and the RC conjugated with the DNA-dye construct all showed similar rates of cytochrome \(c\) oxidation (Figure 4.7A). Apparently, DNA conjugation does not hinder the rate of cytochrome electron transfer to the RC, at least at these concentrations. Moreover, upon 650 nm excitation (Cy5 excitation peak), the DNA-dye conjugated RC showed a much faster rate of oxidation then did the Cys-modified RC or wild type RC, both of which have very low absorbance at 650 nm (Figure 4.7B). Under these conditions, the oxidation rate of cytochrome \(c\) depends on the
Figure 4.7. Cytochrome c oxidation monitored at 550 nm (where the difference in absorbance between reduced and oxidized cytochrome c is maximal) after exciting the RC directly at 800 nm (A) or Cy5 directly at 650 nm (B). Each of the samples shows very similar kinetics upon excitation at 800 nm, which indicates that the presence of the DNA structure on the RC does not affect the ability of cytochrome c to transfer electrons to the RC, at least when the cytochrome is in excess. In contrast, excitation at 650 nm where Cy5 absorbs, results in a rate of cytochrome c oxidation that depends on the number of Cy5 molecules (and thus the absorption cross-section at 650 nm) associated with the complex, with the rate for 3CC being greatest.

number of dye molecules in the construct. This presumably results from the enhanced absorbance cross-section of the light harvesting antenna that increases the number of photons absorbed per unit time by the 3arm DNA-dye-RC complex. Since the spectrum of reduced cytochrome c overlaps strongly with that of Cy3, similar measurements using 550 nm excitation were not attempted as they would have been ambiguous. In the natural system, the RC operates in conjunction with the cytochrome bc₁ complex, cytochrome c₂,
and a quinone pool, to convert light energy into a proton motive force.\textsuperscript{13} In this process, the oxidized initial electron donor of the RC, P\textsuperscript{+}, that is formed upon light-driven electron transfer is subsequently reduced by cytochrome \textit{c}\textsubscript{2}, which docks to the periplasmic face (P side) of the RC. In our artificial antenna system, the 3arm-DNA structures are located on the P side of RC, and so one might expect that this conjugation of DNA close to the docking site of cytochrome would hinder cytochrome binding as well as the electron transfer process from cytochrome to P\textsuperscript{+}. To explore this possibility, a 10-fold molar excess of reduced cytochrome \textit{c}\textsuperscript{14} and a 100-fold molar excess of decylubiquinone were added into a solution of 3arm DNA-dye-RC constructs, and the absorbance intensity change at 550 nm (an absorbance decrease at this wavelength reflects the oxidation of cytochrome \textit{c}) was measured, while either exciting the RC directly or the dye directly.\textsuperscript{9,15} Using 800 nm excitation (direct excitation of the RC), where both the Cy3 and Cy5 have no absorbance, the wild type RC, the Cys-modified RC, and the RC conjugated with the DNA-dye construct all showed similar rates of cytochrome \textit{c} oxidation (Figure 4.7A). Apparently, DNA conjugation does not hinder the rate of cytochrome electron transfer to the RC, at least at these concentrations. Moreover, upon 650 nm excitation (Cy5 excitation peak), the DNA-dye conjugated RC showed a much faster rate of oxidation than did the Cys-modified RC or wild type RC, both of which have very low absorbance at 650 nm (Figure 4.7B). Under these conditions, the oxidation rate of cytochrome \textit{c} depends on the number of dye molecules in the construct. This presumably results from the enhanced absorbance cross-section of the light harvesting antenna that increases the number of photons absorbed per unit time by the 3arm DNA-dye-RC complex. Since the spectrum of reduced cytochrome
c overlaps strongly with that of Cy3, similar measurements using 550 nm excitation were not attempted as they would have been ambiguous.

4.5. Conclusion

A DNA nanostructure with dyes attached at specific positions was conjugated to a RC to serve as a geometrically defined light harvesting antenna. This extended the absorbance cross section of the complex into a spectral range where the RC has only weak absorbance. A combination of placement, spectral properties and excited state kinetic properties of the dyes used are important in determining the efficiency of the antenna in energy transfer. At low light flux, the rate of photon capture by the complex is proportional to the number of dye molecules in the complex that absorb at the excitation wavelength; thus increasing the number of DNA-dye constructs attached to the reaction center increases the functional cross section but does not greatly change the energy transfer efficiency. The complexes explored in this work provide useful model systems for future applications in nanophotonics.

4.6. References


Chapter 5
Summary and Outlook

5.1. Conclusions

After 30 years of Nadrian Seeman’s proposal to create migrationally immobile junctions, which led to the evolution of DNA Nanotechnology, the field is thriving. In general, scientists used to consider DNA as a genetic material, but the idea of constructing nanostructures using DNA was revolutionary. The very basic principle in creating DNA nanostructures is based on ‘Watson-Crick’ base pairing. In last three decades, an enormous amount of work and effort has been put in the field toward various directions, and DNA nanotechnology has already evolved into a multi-disciplinary field.

One of the exciting directions in this context is the self-assembly of many functional materials, including nanoparticles, quantum dots, carbon nanotubes, proteins, virus capsids, and fluorophores using DNA directed self-assembly. Particularly, organic fluorophores have been conjugated covalently to DNA or intercalated to DNA in the development of photonic devices. In this dissertation we have shown the applicability of DNA nanotechnology toward the construction of light-harvesting antenna.

First, we have created multichromophoric seven helix DNA bundles with cyclic arrays of three distinct chromophores. The energy transfer efficiency and the antenna effect (light-harvesting ability) of the triads with different relative ratios of fluorophores were evaluated. Our study showed that with an increase in initial absorption by increasing the number of initial donors, the light-harvesting ability increases. A step-wise energy transfer from the initial donor to the final acceptor via the intermediate donor is confirmed upon analyzing the time-resolved fluorescence.
One important next step is utilization of the excitation energy funneled by artificial light-harvesting antennas and converting it to chemical energy. The bacterial photosynthetic reaction center (RC) is one of the best candidates for this purpose. An obvious approach is to combine the photonic DNA nanostructure and the RC protein, which may act as the both the energy acceptor and the energy transducer. Before this is achieved, it is important to study the direct effects of fluorophores on the properties of the RC. In the chapter 3, we have discussed the effect of three dyes, Alexa Fluor 647, -660 and -750 on the RC in terms of their energy transfer efficiency to RC and formation of the charge-separated state in RC. We found that the energy transfer efficiency is not only dependent on spectral overlap between the dye molecule and RC, but also on the quantum yield and lifetime of the dye molecule used. Enhancement in formation of the charge-separated state of RC in the presences of the dye molecules is due to the enhanced light absorption cross-section offered by the dye molecules that fills the weak absorption region of the RC.

These results led to the third step, which is the conjunction of photonic DNA nanostructure to RC that is discussed in the chapter 4. Simple 3arm-DNA nanostructures conjugated with multiple dyes were utilized as the light-harvesting system. The ratio of the multi-chromophoric 3arm-DNA structure to RC was varied to study the energy transfer efficiencies to the RC and the electron transfer between RC and cytochrome c. Enhanced formation of the charge-separated state in RC is also observed. It is worth mentioning that the DNA structures on the RC do not block the cytochrome c binding site to the RC and the electron transfer process, and the cytochrome c oxidation rate is proportional to the number of dyes on the DNA nanostructure.
This series of studies will provide guidance for future design of complex photonic nano-devices.

5.2. Future Perspective

The need for sustainable and renewable energy sources has driven scientists to mimic the natural photosynthetic systems and create sophisticated artificial devices to utilize the abundant solar energy for mankind. Supramolecular structures, proteins, and other materials have been explored in creating artificial light-harvesting antenna systems. The applicability of DNA nanotechnology toward such goal is emerging. The addressability of DNA nanostructures to organize functional materials in nanometer precision is programmable and promising. Keeping that in mind, inventing future nano-devices with more complex features is the goal of many researchers around the world. One immediate path should be toward the creation of more complex light-harvesting antennas with a huge number of different kinds of fluorophores using DNA as a template in conjunction with RC, which will convert the light energy to chemical energy by charge separated state formation (Figure 5.1). Figure 5.2 shows the schematic representation of a multicomponent nano-device, which can absorb light energy and transfer the energy to a photonic element through light-harvesting systems that will eventually convert the light energy into chemical energy. The RC has limited energy efficiency in terms of light energy-chemical energy conversion. Another approach is to design an artificial RC as the energy transducer, which converts light energy to chemical potential in the form of long-lived charge separation.1 Other than applications in electrical energy production, such a device has huge potential in light driven bio-catalysis.
The advance of scientific knowledge will lead us to future smart devices in which artificial light-harvesting antennas are linked to artificial reaction centers.

**Figure 5.1.** Schematic of 2D DNA light-harvesting antenna array with RC. Grey colored cylinder represents duplex DNA. The DNA 2D array assembles different fluorophores (red and green) and RC (uneven pink structure) in a programmable manner.

**Figure 5.2.** Schematic representation of a photonic device made by organizing multiphotonic elements on DNA template. Reproduced from Reference 1. Copyright 2011 Macmillan Publishers Limited.
5.3. References


Bibliography

Chapter 1 References


Chapter 2 References


(32) The chemical structure of AF was taken from: Chiuman, W.; Li, Y. Nucleic Acids Research 2007, 35, 401.


Chapter 3 References


Chapter 4 References


Chapter 5 References


APPENDIX A

SUPPORTING INFORMATION FOR CHAPTER 2
DNA Directed Artificial Light Harvesting Antenna

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Table S2.1. The calculated quantum yield (QY) of dyes. The numbers were obtained for the individual dyes attached to ssDNA, dsDNA and the 7HB, respectively.

<table>
<thead>
<tr>
<th></th>
<th>ssDNA</th>
<th>dsDNA</th>
<th>7HB (100)</th>
<th>7HB (010)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Py</td>
<td>0.12</td>
<td>0.13</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Cy3</td>
<td>0.28</td>
<td>0.31</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>

Fluorescence Anisotropy: The anisotropy data were measured using a Nanolog, Horiba Jobin Yvon instrument operated in single point anisotropy mode. All of the samples were excited at a particular wavelength (380 nm for Py, 500 nm for Cy3, and 620 nm for AF) and the emission was taken at each respective emission maximum (438 nm for Py, 566 nm for Cy3, and 668 nm for AF) with different excitation and emission polarizer angles, VV, VH, HV and HH. The anisotropy values were calculated using the following equation:

\[ r = \frac{I_W - GI_W}{I_W + 2GI_W} \]

where G, the “G factor”, is

\[ G = \frac{I_{HV}}{I_{HH}} \]
The values are listed below and suggest that all the fluorophores are fairly rigid with limited rotational flexibility in the DNA structure.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Anisotropy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Py (100)</td>
<td>0.27</td>
</tr>
<tr>
<td>Cy3 (010)</td>
<td>0.29</td>
</tr>
<tr>
<td>AF (001)</td>
<td>0.20</td>
</tr>
</tbody>
</table>

**Figure S2.1.** 5% Nondenaturing PAGE gel. (Left) Ethidium bromide stained gel image. (Middle) Typhoon™ Trio multifunction imager (Amersham Biosciences) gel image by Cy3 excitation. (Right) Typhoon gel image by AF excitation. (A), (B), (C), (D) and (E) represent the purified T4, T1, T2, T3 and 100 bp DNA ladder, respectively.

**Figure S2.2.** Structure of 7HB with the sequences of the strands shown.
**Figure S2.3.** Structure of T1 in which positions of the chromophores are indicated with arrows.

**Figure S2.4.** Structure of T2 in which positions of the chromophores are indicated with arrows.

**Figure S2.5.** Structure of T3 in which positions of the chromophores are indicated with arrows.
Figure S2.6. Structure of T4 in which positions of the chromophores are indicated with arrows.

Figure S2.7. (A) Side view, (B) front view and (C) rear view of T1 (6 Py, 6 Cy3 and 1 AF). The typical inter-dye distances between Py and Cy3 are labeled.
Figure S2.8. (A) Structure of T1 in which all the chromophores are denoted by numbers.

The numbers 1, 2, 3, 4, 5 and 6 represent Py; 1’, 2’, 3’, 4’, 5’ and 6’ represent Cy3; and number 0 represents AF. (B) The distances between fluorophores (in nm). Here “1’-0” means distance between 1’ and 0.
Table S2.2. Sequences of the unmodified DNAs for 7HB.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>No. of bases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5'-GTTCTGGTGTAAGCTTGCTTCTGCTGATCGATACTCGCTTATTAGAGTAGAGCTACGAGAGCTGCTATTGTCATAGTACGAG-3'</td>
<td>83</td>
</tr>
<tr>
<td>2</td>
<td>5'-TACATTCGCTTACACGACACACATGCTATGTGTATACCTGACACACACATGCTATGTAAGAGATAGTCCTGTCATATTAGAGTAGAGCTACGAGAGCTGCTATTGTCATAGTACGAG-3'</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>5'-TGCGAAACTCGTAACCTAACGAGGAGGTACGCTACACAGCTCAACCTGACACACATGCTATGTAAGAGATAGTCCTGTCATATTAGAGTAGAGCTACGAGAGCTGCTATTGTCATAGTACGAG-3'</td>
<td>63</td>
</tr>
<tr>
<td>4</td>
<td>5'-CTTTATAAGCTGAGACTGGCAAATGAATCTGACATGGAGTTGCTAAGTTACGA-3'</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>5'-TCGCACTGTGACTGTAACCTGACACACATGCTATGTAAGAGATAGTCCTGTCATATTAGAGTAGAGCTACGAGAGCTGCTATTGTCATAGTACGAG-3'</td>
<td>63</td>
</tr>
<tr>
<td>6</td>
<td>5'-TAATAAAAGAGATGAGACGAGACACACTGAAATGCTACTACGACACACTGACACACATGCTATGTAAGAGATAGTCCTGTCATATTAGAGTAGAGCTACGAGAGCTGCTATTGTCATAGTACGAG-3'</td>
<td>62</td>
</tr>
<tr>
<td>7</td>
<td>5'-CCGAGATTTGCGAGCTACCTGACACACTGACACACATGCTATGTAAGAGATAGTCCTGTCATATTAGAGTAGAGCTACGAGAGCTGCTATTGTCATAGTACGAG-3'</td>
<td>28</td>
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<tr>
<td>8</td>
<td>5'-GATGTTGCTACTGACCGACACTGACACACTGACACACATGCTATGTAAGAGATAGTCCTGTCATATTAGAGTAGAGCTACGAGAGCTGCTATTGTCATAGTACGAG-3'</td>
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<tr>
<td>9</td>
<td>5'-ATTATAATAGCGCTGTGCGACTGCGACATGGACACTACCTGACACACTGACACACATGCTATGTAAGAGATAGTCCTGTCATATTAGAGTAGAGCTACGAGAGCTGCTATTGTCATAGTACGAG-3'</td>
<td>35</td>
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<tr>
<td>10</td>
<td>5'-ATTACCTTGCGCTACTGACACACATGCTATGTAAGAGATAGTCCTGTCATATTAGAGTAGAGCTACGAGAGCTGCTATTGTCATAGTACGAG-3'</td>
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<td>5'-GTAAAGGCAGATAAGGAGGGTGCTATGTAAGAGATAGTCCTGACACACTGACACACATGCTATGTAAGAGATAGTCCTGTCATATTAGAGTAGAGCTACGAGAGCTGCTATTGTCATAGTACGAG-3'</td>
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<td>12</td>
<td>5'-TTAACGATATGCGTGGCTCGAGCCAGATCGGATCTGCGATGCGAATCTGACCAACTCTACAGGCGAGG-3'</td>
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<td>13</td>
<td>5'-CCACGCTGATGTGCGCTGATGCGAATCTGACCAACTCTACAGGCGAGG-3'</td>
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<td>14</td>
<td>5'-GATTGTGCTGTGCGACTGCGACATGGACACTACCTGACACACTGACACACATGCTATGTAAGAGATAGTCCTGTCATATTAGAGTAGAGCTACGAGAGCTGCTATTGTCATAGTACGAG-3'</td>
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<td>15</td>
<td>5'-ATACACTGGTCTGCGACACTGACACACTGACACACATGCTATGTAAGAGATAGTCCTGTCATATTAGAGTAGAGCTACGAGAGCTGCTATTGTCATAGTACGAG-3'</td>
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<td>16</td>
<td>5'-CCACGCTGATGTGCGACTGCGACACTGACACACTGACACACATGCTATGTAAGAGATAGTCCTGTCATATTAGAGTAGAGCTACGAGAGCTGCTATTGTCATAGTACGAG-3'</td>
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<td>17</td>
<td>5'-CTATAAAATAGCGCTGATGCGAATCTGACCAACTCTACAGGCGAGG-3'</td>
<td>63</td>
</tr>
<tr>
<td>18</td>
<td>5'-CTTGGCAATGTCCACCATTGGATCGTGG-3'</td>
<td>28</td>
</tr>
<tr>
<td>19</td>
<td>5'-GTTACACACCCATCGAGCTGCGACTGCGACTGCGACATGGACACTACCTGACACACTGACACACATGCTATGTAAGAGATAGTCCTGTCATATTAGAGTAGAGCTACGAGAGCTGCTATTGTCATAGTACGAG-3'</td>
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Table S2.3. Sequences of the chromophore modified DNAs.

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<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
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</thead>
<tbody>
<tr>
<td>9</td>
<td>5'-ATTCCy3ATAppATAAGGGCTGCGAGACTGCGACTGCGACATGGACATGAGTTAC-3'</td>
</tr>
<tr>
<td>8</td>
<td>5'-GATGTTGCTGACTGCTACCTGACACACTGACACApTTAATCy3-3'</td>
</tr>
<tr>
<td>4</td>
<td>5'-CCTCy3TAppAAGCCAGACCTATCCTPyTATCy3-3'</td>
</tr>
<tr>
<td>14</td>
<td>5'-GATCy3TGTGCTGCGACTGACCATGCGATGAGTTAC-3'</td>
</tr>
<tr>
<td>9</td>
<td>5'-ATTCCy3ATATAGCGCTGTGCGACTGCGACTGCGACATGGACATGAGTTAC-3'</td>
</tr>
<tr>
<td>4</td>
<td>5'-CCTCy3TATAppAAGCCAGACCTATCCTPyTATCy3-3'</td>
</tr>
<tr>
<td>14</td>
<td>5'-GATCy3TGTGCTGCGACTGACCATGCGATGAGTTAC-3'</td>
</tr>
<tr>
<td>8</td>
<td>5'-GATGTTGCTGACTGCTACCTGACACACTGACACApTTATGAC-3'</td>
</tr>
<tr>
<td>9</td>
<td>5'-ATTATAppATAAGGGCTGCGAGACTGCGACTGCGACATGGACATGAGTTAC-3'</td>
</tr>
<tr>
<td>4</td>
<td>5'-CCTTAppAAGCCAGACCTATCCTPyTATTTA-3'</td>
</tr>
<tr>
<td>14</td>
<td>5'-GATGTTGCTGCGACTGACCATGCGATGAGTTAC-3'</td>
</tr>
<tr>
<td>2</td>
<td>5'-AF647-TACTATGGCCTACACGACAC-3'</td>
</tr>
</tbody>
</table>
**Figure S2.9.** Normalized absorption (solid line) and emission (dotted line) spectra for Py (blue), Cy3 (green), and AF (pink) modified ssDNAs showing the optical spectral overlap.
Figure S2.10. Absorption spectra of (A) D1, (B) D2 (Py: Cy3: AF=6:0:1), (C) T1, (D) T2, (E) T3 and (F) T4.
Figure S2.11. Fluorescence spectra of (A) D1, (B) D2, (C) T1, (D) T2, (E) T3 and (F) T4. Peaks at 438 nm, 566 nm and 668 nm correspond to Py, Cy3 and AF emission, respectively ($\lambda_{\text{ex}} = 380$ nm).

Figure S2.12. Fluorescence spectra of (A) T1, (B) T2, (C) T3 and (D) T4. Peaks at 566 nm and 668 nm correspond to Cy3 and AF emission, respectively ($\lambda_{\text{ex}} = 500$ nm).
Figure S2.13. Fluorescence spectra of (A) D2, (B) T1, (C) T2, (D) T3 and (E) T4. Peak at 668 nm corresponds to AF emission ($\lambda_{ex} = 620$ nm).
**Figure S2.14**: From left to right in each row are the following streak camera data: 3D angled view, top view, and integrated spectra over time. The time ranges from 0 to 1.9 ns and the wavelength ranges from 430 nm to 680 nm (with $\lambda_{ex}$=370 nm). The color gradient from red to blue represents decreasing intensity. (A) D1 (6:0:0), (B) sample 1:0:0, (C) T1 (6:6:1), (D) T2 (6:3:1), (E) T3 (3:6:1) and (F) T4 (1:1:1). The red dotted lines in the integrated spectral data represents the sensitivity corrected spectra in the range of 570-680 nm, using the manufacture provided wavelength dependent sensitivity data of the streak tube (10 nm intervals) normalized at 570 nm. After the correction, the dynamic spectral data (integrated data) closely resembles the steady state spectra shown in Figure 4 and Figure S11. The dim line in the 460-480 nm spectral region (more obvious in panel A) was an instrument artifact, which was later corrected as shown in panel B.

![Streak Camera Data](image.png)

**Figure S2.15.** Fluorescence decay profile of ssDNA using a Streak camera. Py labeled ssDNA (5’-ATTATA*Py*ATAGCGTCTGCGACTGGCA-TGTGATAC-3’), D1 and T1–T4 monitored at 460 nm ($\lambda_{ex}$ = 370 nm). This data can be compared with the TCSPC...
data shown in Figure 5, although this data contains more noise, both show the same trend in the lifetime change.

**Figure S2.16.** Time resolved emission of T1 monitored at (A) 460 nm (Py decay), (B) 560 nm (Cy3 decay) and (C) 660 nm (AF decay) using streak camera ($\lambda_{ex} = 370$ nm). Red lines represent the fitted data.

**Figure S2.17.** Time resolved emission of T2 monitored at (A) 460 nm (Py decay), (B) 560 nm (Cy3 decay) and (C) 660 nm (AF decay) using streak camera ($\lambda_{ex} = 370$ nm). Red lines represent the fitted data.
Figure S2.18. Time resolved emission of T3 monitored at (A) 460 nm (Py decay), (B) 560 nm (Cy3 decay) and (C) 660 nm (AF decay) using streak camera (λ<sub>ex</sub> = 370 nm). Red lines represent the fitted data.

Figure S2.19. Time resolved emission of T4 monitored at (A) 460 nm (Py decay), (B) 560 nm (Cy3 decay) and (C) 660 nm (AF decay) using streak camera (λ<sub>ex</sub> = 370 nm). Red lines represent the fitted data.

Figure S2.20. Decay associated spectra (DAS) of (A) 100, (B) 010, (C) 001 (100, 010 and 001 represent 7HB each containing a single Py, Cy3, and AF, respectively, which are
structurally similar to the triads used in this study). Excitation wavelengths are 370 nm, 500 nm and 620 nm respectively.

**Figure S2.21.** DAS of Py in (A) ssDNA and (B) D1 ($\lambda_{ex} = 370$ nm). No obvious difference in fluorescein dynamics is observed when Py is incorporated in ssDNA, dsDNA, singly labeled on the 7HB, or 6 Py labeled on DNA, except for slight variations in the observed lifetimes.

**Figure S2.22.** DAS of (A) Cy3 in ssDNA, and (B) AF in ssDNA. Excitation wavelengths are 500 nm and 620 nm, respectively. Comparing the data shown in Figure S20B and (A) here, the fluorescence dynamics of Cy3 shows some structural dependence. The decay is slightly faster when Cy3 is on ssDNA than when it is on the 7HB DNA nanostructure. AF exhibits little structural dependence.
**Figure S2.23.** DAS of (A) T1, (B) T2, (C) T3, and (D) T4 (highlighting only the AF emission region).

**Figure S2.24.** Normalized DAS of T1 to compare the spectral features of the two longer lifetime components ($\lambda_{ex} = 370$ nm). The later spectrum shows an obvious increase in intensity in the AF emission region, illustrating that AF is the acceptor of the energy transfer. The intensity in this spectral region should be about 2 folds higher than it appears as the sensitivity of the streak tube drops dramatically here.
Figure S2.25. Characterization of 5’-ATTCy3ATAPyATAGCGTCTGCGACTGGC
ATGTGATAC-3’. HPLC (left) and MALDI-TOF-MS (right). In HPLC, the upper trace,
middle trace and lower trace correspond to absorption at 260, 380 and 540 nm, respectively.
Mass calculated: 11511.3; mass observed: 11513.7 (z = 1).

Figure S2.26. Characterization of 5’-GATGTGTGCTACTTGCACCACTGTACAPy
TTACy3TAG-3’. HPLC (left) and MALDI-TOF-MS (right). In HPLC, the upper trace,
middle trace and lower trace correspond to absorption at 260 nm, 380 nm and 540 nm,
respectively. Mass calculated: 11441.80; mass observed: 11443.7 (z = 1).
Figure S2.27. Characterization of 5’-CTT<sub>Cy3</sub>TATPyAAGCAGACCTATCTPyTA-TCy3TTA-3’. HPLC (left) and MALDI-TOF-MS (right). In HPLC, the upper trace, middle trace and lower trace correspond to absorption at 260 nm, 380 nm and 540 nm, respectively. Mass calculated: 9938.4; mass observed: 9940.1 (z = 1).

Figure S2.28. Characterization of 5’-GAT<sub>Cy3</sub>TGTPyTGTGCAACCGATCAPyTCTCy3-TAA-3’. HPLC (left) and MALDI-TOF-MS (right). In HPLC, the upper trace, middle trace and lower trace correspond to absorption at 260 nm, 380 nm and 540 nm, respectively. Mass calculated: 10004.4; mass observed: 10006.5 (z = 1).
**Figure S2.29.** Characterization of 5’-ATT<sup>Cy3</sup>TATATAGCGTCGTGCGACTGGC ATGTGATAC-3’. HPLC (left) and MALDI-TOF-MS (right). In HPLC, the upper trace and lower trace correspond to absorption at 260 nm and 540 nm, respectively. Mass calculated: 11307.4; mass observed: 11308.5 (z = 1).

**Figure S2.30.** Characterization of 5’-CTT<sup>Cy3</sup>TATTAAGCAGACCTCTPyTAT<sup>Cy3</sup>-TTA-3’. HPLC (left) and MALDI-TOF-MS (right). In HPLC, the upper trace, middle trace and lower trace correspond to absorption at 260 nm, 380 nm and 540 nm, respectively. Mass calculated: 9726.0; mass observed: 9727.1 (z = 1).
Figure S2.31. Characterization of 5’-GAT\text{Cy3}TGGTTGTGCAACCGATCAPyTCT\text{Cy3}-TAA-3’. HPLC (left) and MALDI-TOF-MS (right). In HPLC, the upper trace, middle trace and lower trace correspond to absorption at 260 nm, 380 nm and 540 nm, respectively. Mass calculated: 9792.0; mass observed: 9795.4 (z = 1).

Figure S2.32. Characterization of 5’-CCT\text{Cy3}TATPyAAGCAGACCTATCTPyTAT TTA-3’. HPLC (left) and MALDI-TOF-MS (right). In HPLC, the upper trace, middle trace and lower trace correspond to absorption at 260 nm, 380 nm and 540 nm, respectively. Mass calculated: 9129.9; mass observed: 9128.6 (z = 1).
Figure S2.33. Characterization of 5’-GAT\textsubscript{Cy3}TG\textsubscript{Py}TG\textsubscript{PY}TGTGCAACGATCAP\textsubscript{Py}TCT TAA-3’. HPLC (left) and MALDI-TOF-MS (right). In HPLC, the upper trace, middle trace and lower trace correspond to absorption at 260 nm, 380 nm and 540 nm, respectively. Mass calculated: 9168.9; mass observed: 9189.8 (z = 1).

Figure S2.34. Characterization of 5’-GATGTGTGCTACTTGTCAACCACGTACAG\textsubscript{Py}T TATAG-3’. HPLC (left) and MALDI-TOF-MS (right). In HPLC, the upper trace and lower trace correspond to absorption at 260 nm and 380 nm, respectively. Mass calculated: 11934.2; mass observed: 11939.1 (z = 1).
Figure S2.35. Characterization of 5’-ATTATA\textsubscript{Py}ATAGCGTCGTGGCACTGGCATG-TGATAC-3’. HPLC (left) and MALDI-TOF-MS (right). In HPLC, the upper trace and lower trace correspond to absorption at 260 nm and 380 nm, respectively. Mass calculated: 11008.3; mass observed: 11009.0 (z = 1).

Figure S2.36. Characterization of 5’-CTTTAT\textsubscript{Py}AAGCAGACCTATCT\textsubscript{Py}TATTTA-3’. HPLC (left) and MALDI-TOF-MS (right). In HPLC, the upper trace and lower trace correspond to absorption at 260 nm and 380 nm, respectively. Mass calculated: 8923.3; mass observed: 8945.1 (z = 1).
Figure S2.37. Characterization of 5’-GATTGTPyTGTGCAACCGATCAPyTCTTAA-3’. HPLC (left) and MALDI-TOF-MS (right). In HPLC, the upper trace, and lower trace correspond to absorption at 260 nm, and 380 nm, respectively. Mass calculated: 8989.2; mass observed: 8998.8 (z = 1).
APPENDIX B

SUPPORTING INFORMATION FOR CHAPTER 3
Reengineering the Optical Absorption Cross-section of Photosynthetic Reaction Centers

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1Department of Chemistry and Biochemistry and 2The Biodesign Institute, Arizona State University, Tempe, AZ 85287, United States; 3Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, V6T 1Z3, Canada.

Figure S3.1. MALDI-TOF spectra of the RC (A), RC-AF660 (B) and together (smoothed) (C). The RC spectrum shows three peaks at 28960, 31395 and 34554 m/Z, corresponding to the H, L and M subunits, respectively. In contrast, the Alexa Fluor 660 conjugate (the center spectrum) has two additional peaks (32260 and 33066 m/Z), roughly 865 and 1671 Daltons different from that of the L subunit, corresponding to L subunits bound to one and two dye molecules. The peak of the M subunit has a shoulder at 35474 m/Z, differing by roughly 900 Daltons from that of the M subunit, corresponding to one dye molecule per M subunit. This result confirms the selective conjugation of the dye to the mutated RC, in which the L subunit has two surface cysteine residues and the M subunit has one surface cysteine, while the H subunit lacks cysteine.
Figure S3.2. A, B and C are the absorption spectra (left) and fluorescence spectra (right) of AF647, BSA-AF647 and RC-AF647, respectively, with the corresponding absorbance and emission maxima marked.
Figure S3.3. A, B and C are the absorption spectra (left) and fluorescence spectra (right) of AF660, BSA-AF660 and RC-AF660, respectively, with the corresponding absorbance and emission maxima marked.
Figure S3.4. A, B and C are the absorption spectra (left) and fluorescence spectra (right) of AF750, BSA-AF750 and RC-AF750, respectively, with the corresponding absorbance and emission maxima marked.
Figure S3.5. (Left to right) Absorption spectra of RC (purple) and RC conjugated with Alexa Fluor dye (black), fluorescence emission spectra of Alexa Fluor dye conjugated to BSA (black) and to RC (red), and time resolved fluorescence decay profile of Alexa Fluor dye conjugated to BSA (blue) and RC (purple) using time correlated single photon counting. The upper row data are for Alexa Fluor 647 (excitation wavelength 600 nm, decay kinetics monitored at 670 nm), the middle row data are for Alexa Fluor 660 (excitation wavelength 600 nm, decay kinetics monitored at 698 nm) and the bottom row data are for Alexa Fluor 750 (excitation wavelength 710 nm, decay kinetics monitored at 780 nm).
Figure S3.6. A, B and C are the fluorescence life-time decay profile of free dye AF647, AF660 and AF750, respectively, in tris buffer (15 mM tris, pH 8, 0.025% LDAO, 150 mM NaCl, 1 mM EDTA). Biexponential decay kinetics was used for fitting all of the three dyes. The fitting parameters are shown in Table S2.

Table S3.1. Absorption, and fluorescence maxima and quantum yields for all the samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorption maxima (nm)</th>
<th>Emission Maxima (nm)</th>
<th>Quantum Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF647</td>
<td>Free dye</td>
<td>649</td>
<td>667</td>
</tr>
<tr>
<td></td>
<td>On BSA</td>
<td>654</td>
<td>674</td>
</tr>
<tr>
<td></td>
<td>On RC</td>
<td>654</td>
<td>674</td>
</tr>
<tr>
<td>AF660</td>
<td>Free dye</td>
<td>661</td>
<td>690</td>
</tr>
<tr>
<td></td>
<td>On BSA</td>
<td>665</td>
<td>697</td>
</tr>
<tr>
<td></td>
<td>On RC</td>
<td>665</td>
<td>697</td>
</tr>
<tr>
<td>AF750</td>
<td>Free dye</td>
<td>752</td>
<td>780</td>
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<tr>
<td></td>
<td>On BSA</td>
<td>762</td>
<td>784</td>
</tr>
<tr>
<td></td>
<td>On RC</td>
<td>762</td>
<td>790</td>
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Table S3.2. Fitting parameters of the life-time data for all the samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>τ1 ns (amplitude %)</th>
<th>τ2 ns (amplitude %)</th>
<th>τ3 ns (amplitude %)</th>
<th>Average lifetime (ns)</th>
<th>κ^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF647</td>
<td>0.41 (6.9)</td>
<td>1.09 (93.1)</td>
<td>-</td>
<td>1.04</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>Free BSA RC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05 (30.8)</td>
<td>0.64 (19.8)</td>
<td>1.73 (49.4)</td>
<td>0.99</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.07 (62.9)</td>
<td>1.61 (20.9)</td>
<td>0.45</td>
<td>1.04</td>
</tr>
<tr>
<td>AF660</td>
<td>0.55 (19)</td>
<td>1.24 (81)</td>
<td>-</td>
<td>1.11</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>Free BSA RC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.10 (13.2)</td>
<td>0.81 (25.5)</td>
<td>1.82 (61.3)</td>
<td>1.33</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.04 (67.9)</td>
<td>1.46 (16.0)</td>
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<td>1.08</td>
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<td></td>
<td>Q^- RC</td>
<td>0.05 (60.3)</td>
<td>0.47 (18.9)</td>
<td>0.45</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.60 (20.8)</td>
<td></td>
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<tr>
<td>AF750</td>
<td>0.55 (98.6)</td>
<td>1.17 (1.4)</td>
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<td>0.56</td>
<td>1.02</td>
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<tr>
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<td>Free BSA RC</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>0.12 (11.7)</td>
<td>0.73 (76.0)</td>
<td>1.63 (12.3)</td>
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<td>1.16</td>
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<tr>
<td></td>
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<td>0.06 (40.0)</td>
<td>0.85 (25.4)</td>
<td>0.37</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Figure S3.7. Normalized transient absorbance of AF660 (red) in QdelRC-AF660, and P^+ in QdelRC-AF660 (blue) and in the unconjugated QdelRC (green). Excitation was at 650 nm for all samples.
Table S3.3. Fitting parameters for the pump-probe data (pump at 650 nm and probe at 700 nm).

<table>
<thead>
<tr>
<th>Sample</th>
<th>τ1 ps (amplitude %)</th>
<th>τ2 ps (amplitude %)</th>
<th>τ3 ps (amplitude %)</th>
<th>Average (ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF660</td>
<td>519 (30%)</td>
<td>1390 (70%)</td>
<td>-</td>
<td>1129</td>
</tr>
<tr>
<td>BSA-AF660</td>
<td>531 (18%)</td>
<td>1800 (82%)</td>
<td>-</td>
<td>1572</td>
</tr>
<tr>
<td>QdelRC-AF660</td>
<td>25 (35%)</td>
<td>164 (29%)</td>
<td>1418 (36%)</td>
<td>567</td>
</tr>
</tbody>
</table>

Table S3.4. Comparison of energy transfer (ET) efficiency calculated from steady-state fluorescence, fluorescence lifetime decay and transient absorbance with corresponding dye on BSA as control.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ET from steady-state fluorescence (%)</th>
<th>ET from fluorescence lifetime measurement (%)</th>
<th>ET from transient absorbance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RC-AF647</td>
<td>59</td>
<td>55</td>
<td>NA</td>
</tr>
<tr>
<td>RC-AF660</td>
<td>71</td>
<td>77</td>
<td>NA</td>
</tr>
<tr>
<td>QdelRC-AF660</td>
<td>70</td>
<td>66</td>
<td>64</td>
</tr>
<tr>
<td>RC-AF750</td>
<td>60</td>
<td>52</td>
<td>NA</td>
</tr>
</tbody>
</table>

$^1E = 1 - \frac{I_{DA}/A_{DA}}{I_D/A_D}$, Where $I_{DA}$ and $I_D$ are the integrated area of donor fluorescence emissions with and without acceptors. $A_{DA}$ and $A_D$ are the absorbance of donor at excitation wavelength with and without acceptors.

$^2E_{\text{lifetime}} = 1 - \frac{\tau_{ave,DA}}{\tau_{ave,D}}$, where $\tau_{ave,DA}$ and $\tau_{ave,D}$ are the average lifetime of donor with and without acceptors obtained from the fluorescence time-resolved single photon counting measurements.

$^3E_{TA} = 1 - \frac{\tau_{ave,DA}}{\tau_{ave,D}}$, where $\tau_{ave,DA}$ and $\tau_{ave,D}$ are the average lifetime of donor with and without acceptors obtained from the transient absorbance measurements.
**Figure S3.8.** The absorbance in light (with excitation at 650 nm) and in dark for RC only (A), RC-AF660 conjugate (B) and RC-AF647 conjugate (C).

**Figure S3.9.** (A) Time-resolved transient absorption difference spectra of BSA-AF660 in the 530-730 nm region. (B) Transient absorbance kinetics at 700 nm for unconjugated AF660 dye in solution (AF660, blue), quinone-depleted RC-AF660 conjugates (Q_{del}RC-AF660, red) and BSA-AF660 conjugates (BSA-AF660, pink). For all samples, \( \lambda_{ex} = 650 \) nm.
Figure S3.10. Comparison between 1-transmittance and excitation spectra of different samples. For AF660 dye series, the excitation was done from 450 nm to 700 nm and the emission was taken at 710 nm. Whereas for AF647 dye series, the excitation was done from 450 nm to 680 nm and the emission was taken at 690 nm.
APPENDIX C

SUPPORTING INFORMATION FOR CHAPTER 4
A DNA Directed Light-Harvesting/Reaction Center System

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Figure S4.1: Structure of the RC (mutated) from the purple bacterium, Rhodobacter sphaeroides 2.4.1 (PDB 2J8C). The three subunits H (green), M (cyan) and L (red) are shown with all cofactors. Three unique mutated Cys on the surface of the RC close to the P side are shown in red color with their relative distances marked. There are two Cys residues on L and one on M subunit. Details of the cofactors with labeling are shown on left.
**Figure S4.2:** Nondenaturing PAGE image (8%). (Top) Typhoon™ Trio multifunction imager (Amersham Biosciences) gel image by exciting at 532 nm and 633 nm. (Bottom) Ethidium bromide stained gel image. (1), (2), (3) and (4) represent the purified 3arm labeled with Cy3, Cy3-Cy5, AF660 and AF660-AF750, respectively.

**Figure S4.3:** (A) Schematic of 3arm-DNA structure with Cy3 only and with both Cy3 and Cy5. (B)-(C) Absorption spectra of 3arm-Cy3 and 3arm-Cy3-Cy5. (D) Corresponding fluorescence emission spectra with excitation at 510 nm. The spectra were corrected by
detector response file and normalized by dye absorbance at 510 nm. A 78% energy transfer is observed from Cy3 to Cy5.

**Figure S4.4:** (A)-(B) Absorption spectra of 3arm-AF660 and 3arm-AF660-AF750. (C) Corresponding fluorescence emission spectra with excitation at 600 nm. The spectra were corrected by detector response file and normalized by dye absorbance at 600 nm. A 57% energy transfer is observed from AF660 to AF750.
Figure S4.5: Absorbance (left) and fluorescence (right) spectra of RC with different ratio of 3arm-Cy3. Strand-2 and Cy3-labeled Strand-3 were allowed to hybridize to the RC-DNA conjugated with 1 to 3 Strand-1 per RC, respectively, and the actual 3-arm DNA per RC were obtained to be 0.8-2.4 from the absorbance spectra. The energy transfer efficiency (E) values between the Cy3 and RC are marked in the fluorescence spectra.
**Figure S4.6:** (A) Absorbance spectra of RC with 1 copy of 3arm-Cy3-Cy5 (the actual ratio is 0.8 obtained from the absorbance spectrum). (B) Fluorescence spectra of 3arm-Cy3-Cy5-RC compared with that of 3arm-Cy3 when Cy3 is excited ($\lambda_{ex} = 510$ nm). The total energy transfer from Cy3 to Cy5 and RC is 83%. (C) Fluorescence spectra of 3arm-Cy3-Cy5-RC compared with that of 3arm-Cy3-Cy5 when Cy3 is excited ($\lambda_{ex} = 510$ nm). The overall energy transfer from Cy3 and Cy5 to RC is 51%. (D) Fluorescence spectra of 3arm-Cy3-Cy5-RC compared with that of 3arm-Cy3-Cy5 when only Cy5 is excited ($\lambda_{ex} = 620$ nm). The energy transfer from Cy5 to RC is 58%.
Figure S4.7: (A) Absorbance spectra of RC with 2 copies of 3arm-Cy3-Cy5 (the actual ratio is 1.6 obtained from the absorbance spectrum). (B) Fluorescence spectra of 3arm-Cy3-Cy5-RC compared with that of 3arm-Cy3 when Cy3 is excited ($\lambda_{ex} = 510$ nm). The total energy transfer from Cy3 to Cy5 and RC is 82%. (C) Fluorescence spectra of 3arm-Cy3-Cy5-RC compared with that of 3arm-Cy3-Cy5 when Cy3 is excited ($\lambda_{ex} = 510$ nm). The overall energy transfer from Cy3 and Cy5 to RC is 47%. (D) Fluorescence spectra of 3arm-Cy3-Cy5-RC compared with that of 3arm-Cy3-Cy5 when only Cy5 is excited ($\lambda_{ex} = 620$ nm). The energy transfer from Cy5 to RC is 49%.
**Figure S4.8:** (A) Absorbance spectrum of RC with 3 copies of 3arm-Cy3-Cy5 (the actual ratio is 2.4 obtained from the absorbance spectrum). (B) Fluorescence spectra of 3arm-Cy3-Cy5-RC compared with that of 3arm-Cy3 when Cy3 is excited ($\lambda_{ex} = 510$ nm). The total energy transfer from Cy3 to Cy5 and RC is 84%. (C) Fluorescence spectra of 3arm-Cy3-Cy5-RC compared with that of 3arm-Cy3-Cy5 when Cy3 is excited ($\lambda_{ex} = 510$ nm). The overall energy transfer from Cy3 and Cy5 to RC is 45%. (D) Fluorescence spectra of 3arm-Cy3-Cy5-RC compared with that of 3arm-Cy3-Cy5 when only Cy5 is excited ($\lambda_{ex} = 620$ nm). The energy transfer from Cy5 to RC is 48%.
Figure S4.9: Absorbance (left) and fluorescence (right) spectra of RC with different ratio of 3arm-AF660. The energy transfer efficiency values are shown on the fluorescence spectra (blue color text).
**Figure S4.10:** (A) Absorbance spectrum of RC with 1 copy of 3arm-AF660-AF750 (the actual ratio is 0.8 obtained from the absorbance spectrum). (B) Fluorescence spectra of 3arm-AF660-AF750-RC compared with that of 3arm-AF660 when AF660 is excited ($\lambda_{ex} = 600$ nm). The total energy transfer from AF660 to AF750 and RC is 75%. (C) Fluorescence spectra of 3arm-AF660-AF750-RC compared with that of 3arm-AF660-AF750 when AF660 is excited ($\lambda_{ex} = 600$ nm). The overall energy transfer from AF660 and AF750 to RC is 43%. (D) Fluorescence spectra of 3arm-AF660-AF750-RC compared with that of 3arm-AF660-AF750 when only AF750 is excited ($\lambda_{ex} = 700$ nm). The energy transfer from AF750 to RC is 41%.
Figure S4.11: (A) Absorbance spectrum of RC with 2 copies of 3arm-AF660-AF750 (the actual ratio is 1.6 obtained from the absorbance spectrum). (B) Fluorescence spectra of 3arm-AF660-AF750-RC compared with that of 3arm-AF660 when AF660 is excited (λ_{ex} = 600 nm). The total energy transfer from AF660 to AF750 and RC is 74%. (C) Fluorescence spectra of 3arm-AF660-AF750-RC compared with that of 3arm-AF660-AF750 when AF660 is excited (λ_{ex} = 600 nm). The overall energy transfer from AF660 and AF750 to RC is 47%. (D) Fluorescence spectra of 3arm-AF660-AF750-RC compared with that of 3arm-AF660-AF750 when only AF750 is excited (λ_{ex} = 700 nm). The energy transfer from AF750 to RC is 43%. 
**Figure S4.12:** (A) Absorbance spectrum of RC with 3 copies of 3arm-AF660-AF750 (the actual ratio is 2.1 obtained from the absorbance spectrum). (B) Fluorescence spectra of 3arm-AF660-AF750-RC compared with that of 3arm-AF660 when AF660 is excited ($\lambda_{ex} = 600$ nm). The total energy transfer from AF660 to AF750 and RC is 77%. (C) Fluorescence spectra of 3arm-AF660-AF750-RC compared with that of 3arm-AF660-AF750 when AF660 is excited ($\lambda_{ex} = 600$ nm). The overall energy transfer from AF660 and AF750 to RC is 48%. (D) Fluorescence spectra of 3arm-AF660-AF750-RC compared with that of 3arm-AF660-AF750 when only AF750 is excited ($\lambda_{ex} = 700$ nm). The energy transfer from AF750 to RC is 43%.
Figure S4.13: Time resolved emission of 3arm-Cy3-Cy5 with and without RC. (A) Cy5 emission monitored at 668 nm by exciting Cy3 (510 nm). (B) Cy5 emission monitored at 668 nm by exciting Cy5 (620 nm).

Figure S4.14: Time resolved emission of 3arm-AF660 and 3arm-AF660-AF750 samples with and without RC. (A) AF660 emission monitored at 698 nm by exciting AF660 (600 nm). (B) AF750 emission monitored at 780 nm by exciting AF660 (600 nm). (C) AF750 emission monitored at 780 nm by exciting AF750 (740 nm).
**Table S4.1**: Fitting parameters for the Cy3 lifetime data in different constructs, monitored at 565 nm ($\lambda_{ex} = 510$ nm). 1C to 3C represent Cy3 labeled 3arm DNA conjugated to RC that have 3arm to RC ratio of 1 to 3. 1CC to 3CC represent both Cy3- and Cy5- labeled 3arm-DNA conjugated to RC that have 3arm to RC ratio of 1 to 3. The results from two replicates of each sample are shown.

<table>
<thead>
<tr>
<th>sample</th>
<th>$\tau_1$ ns (amplitude %)</th>
<th>$\tau_2$ ns (amplitude %)</th>
<th>$\tau_3$ ns (amplitude %)</th>
<th>$\tau_4$ ns (amplitude %)</th>
<th>$\chi^2$</th>
<th>average lifetime (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3arm-Cy3</td>
<td>0.63(34.9)</td>
<td>2.41(65.1)</td>
<td></td>
<td></td>
<td>1.18</td>
<td>1.788</td>
</tr>
<tr>
<td></td>
<td>0.64(35.5)</td>
<td>2.45(64.5)</td>
<td></td>
<td></td>
<td>1.17</td>
<td>1.807</td>
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<tr>
<td>3arm-Cy3-Cy5</td>
<td>0.06(59.8)</td>
<td>0.40(22.9)</td>
<td>2.15(17.3)</td>
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<tr>
<td></td>
<td>0.07(52.7)</td>
<td>0.52(23.6)</td>
<td>2.19(23.7)</td>
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<td>1C</td>
<td>0.12(14.1)</td>
<td>0.68(45.0)</td>
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<td>1.059</td>
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<tr>
<td></td>
<td>0.09(12.1)</td>
<td>0.67(42.7)</td>
<td>1.9(45.2)</td>
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<td>0.09(11.5)</td>
<td>0.66(46.5)</td>
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<td>3C</td>
<td>0.10(12.5)</td>
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<tr>
<td></td>
<td>0.11(13.2)</td>
<td>0.75(45.4)</td>
<td>1.96(41.4)</td>
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<td>1.14</td>
<td>1.167</td>
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<tr>
<td>1CC</td>
<td>0.04 (50.9)</td>
<td>0.15(28.7)</td>
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<td>0.03(51.5)</td>
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<td>0.14(27.3)</td>
<td>0.48(11.7)</td>
<td>1.75(7.4)</td>
<td>1.02</td>
<td>0.240</td>
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Table S4.2: Fitting parameters for Cy5 lifetime data, monitored at 668 nm ($\lambda_{ex} = 620$ nm).

The results from two replicates of each sample are shown.

<table>
<thead>
<tr>
<th>sample</th>
<th>$\tau_1$ ns (amplitude %)</th>
<th>$\tau_2$ ns (amplitude %)</th>
<th>$\tau_3$ ns (amplitude %)</th>
<th>$\kappa^2$</th>
<th>average lifetime (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3armCy3-Cy5</td>
<td>0.77(22.8)</td>
<td>1.92(77.2)</td>
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<td>1.658</td>
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<tr>
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<td>0.84(26.2)</td>
<td>1.92(73.8)</td>
<td>1.16</td>
<td>1.637</td>
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<tr>
<td>1CC</td>
<td>0.10(41.9)</td>
<td>0.48(26.7)</td>
<td>1.06</td>
<td>0.763</td>
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<tr>
<td></td>
<td>0.10(36.8)</td>
<td>0.51(24.8)</td>
<td>1.07</td>
<td>0.900</td>
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<tr>
<td>2CC</td>
<td>0.11(40.3)</td>
<td>0.50(25.7)</td>
<td>1.02</td>
<td>0.802</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.10(37.8)</td>
<td>0.48(26.0)</td>
<td>1.03</td>
<td>0.836</td>
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<tr>
<td>3CC</td>
<td>0.11(45.0)</td>
<td>0.45(27.9)</td>
<td>1.03</td>
<td>0.674</td>
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<tr>
<td></td>
<td>0.10(37.5)</td>
<td>0.51(24.5)</td>
<td>1.02</td>
<td>0.873</td>
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Table S4.3: Fitting parameters for AF660 lifetime data, monitored at 698 nm ($\lambda_{ex} = 600$ nm). 1-6 to 3-6 represent AF660 labeled 3arm DNA conjugated to RC that have 3arm to RC ratio of 1 to 3. 1-6-7 to 3-6-7 represent both AF660- and AF750- labeled 3arm-DNA conjugated to RC that have 3arm to RC ratio of 1 to 3. The results from two replicates of each sample are shown.

<table>
<thead>
<tr>
<th>sample</th>
<th>$\tau_1$ ns (amplitude %)</th>
<th>$\tau_2$ ns (amplitude %)</th>
<th>$\tau_3$ ns (amplitude %)</th>
<th>$\kappa^2$</th>
<th>average lifetime (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3arm660</td>
<td>1.10(27.4)</td>
<td>1.90(72.6)</td>
<td>1.09</td>
<td>1.681</td>
<td></td>
</tr>
<tr>
<td>3arm-660-750</td>
<td>0.08(25.5)</td>
<td>0.90(19.1)</td>
<td>1.08</td>
<td>1.123</td>
<td></td>
</tr>
<tr>
<td>1-6</td>
<td>0.61(39.5)</td>
<td>1.70(60.5)</td>
<td>1.06</td>
<td>1.267</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.62(32.4)</td>
<td>1.69(67.6)</td>
<td>1.15</td>
<td>1.343</td>
<td></td>
</tr>
<tr>
<td>2-6</td>
<td>0.65(38.5)</td>
<td>1.73(61.5)</td>
<td>1.11</td>
<td>1.314</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.65(35.6)</td>
<td>1.65(64.4)</td>
<td>1.10</td>
<td>1.294</td>
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<tr>
<td>3-6</td>
<td>0.63(37.6)</td>
<td>1.76(62.4)</td>
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<tr>
<td></td>
<td>0.64(37.2)</td>
<td>1.64(62.8)</td>
<td>1.14</td>
<td>1.268</td>
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<tr>
<td>1-6-7</td>
<td>0.08(36.3)</td>
<td>0.58(25.8)</td>
<td>1.07</td>
<td>0.823</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.09(28.0)</td>
<td>0.68(24.2)</td>
<td>1.04</td>
<td>0.978</td>
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</tr>
<tr>
<td>2-6-7</td>
<td>0.08(33.6)</td>
<td>0.57(25.8)</td>
<td>1.00</td>
<td>0.823</td>
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<tr>
<td></td>
<td>0.09(29.1)</td>
<td>0.65(22.4)</td>
<td>1.07</td>
<td>0.928</td>
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<tr>
<td>3-6-7</td>
<td>0.07(39.3)</td>
<td>0.52(27.1)</td>
<td>1.12</td>
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<td></td>
<td>0.08(36.7)</td>
<td>0.59(23.2)</td>
<td>1.13</td>
<td>0.784</td>
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**Table S4.4:** Fitting parameters for AF750 lifetime data, monitored at 780 nm ($\lambda_{ex} = 740$ nm).

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<thead>
<tr>
<th>sample</th>
<th>$\tau_1$ ns (amplitude %)</th>
<th>$\tau_2$ ns (amplitude %)</th>
<th>$\tau_3$ ns (amplitude %)</th>
<th>$\kappa^2$</th>
<th>average lifetime (ns)</th>
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<tr>
<td>3arm-660-750</td>
<td>0.48(31.1)</td>
<td>0.72(68.9)</td>
<td>1.08</td>
<td>0.645</td>
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</tr>
<tr>
<td>1-6-7</td>
<td>0.08(25.4)</td>
<td>0.56(59.6)</td>
<td>1.04(15.0)</td>
<td>1.07</td>
<td>0.510</td>
</tr>
<tr>
<td>2-6-7</td>
<td>0.07(31.6)</td>
<td>0.46(37.0)</td>
<td>0.81(31.4)</td>
<td>1.15</td>
<td>0.446</td>
</tr>
<tr>
<td>3-6-7</td>
<td>0.08(39.0)</td>
<td>0.47(28.8)</td>
<td>0.78(32.2)</td>
<td>1.19</td>
<td>0.418</td>
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**Figure S4.15:** Energy transfer efficiencies calculated from lifetime data. (A) Energy transfer efficiencies using Cy3 and Cy5 as antenna. Green bars represent the energy transfer efficiencies from Cy3 to RC (for 1C, 2C, 3C), or the sum of Cy3 to Cy5 and Cy3 to RC (for 1CC, 2CC, 3CC). Red bars represent the energy transfer efficiencies between Cy5 and RC. (B) Energy transfer efficiencies using AF660 and AF750 as antenna. Green bars represent the energy transfer efficiencies from AF660 to RC (for 1-6, 2-6, 3-6), or the sum of AF660 to AF750 and AF660 to RC (for 1-6-7, 2-6-7, 3-6-7). Red bars represent the energy transfer efficiencies between AF750 and RC.
Figure S4.16: Transmittance spectra of filters used in the light-minus-dark absorbance spectroscopy measurements, (A) for excitation at 650 nm and (B) for excitation at 800 nm.
**Figure S4.17:** MALDI-TOF spectra of (A) amine modified Strand-3, (B) Alexa Fluor 660 conjugated Strand-3., (C) amine modified Strand-2, (D) AF750 conjugated Strand-2, and (E) amine modified Strand-1.

![MALDI-TOF spectra](image)

**Figure S4.18:** Pump-probe transient absorbance kinetics of RC, probed at 870 nm and pumped at 650 nm. (A) RC alone, and (B) with 3arm-DNA. Both data show ground state bleaching of P. Fitting components with corresponding amplitude values are also shown. The slow rise with a 36 ps component having negative amplitude in the case of RC with 3arm clearly shows the slow increase in P* formation, which is due to the energy transfer from the fluorophore.

![Pump-probe transient absorbance kinetics](image)
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Publication: Journal of the American Chemical Society
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Publication: Journal of the American Chemical Society
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Publication: Journal of the American Chemical Society

Publisher: American Chemical Society

Date: March 1, 2011

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