DESIGN, SYNTHESIS, AND CHARACTERIZATION OF
NANOSCALE OPTICAL DEVICES USING DNA DIRECTED SELF-ASSEMBLY

by

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The following individuals read and discussed the dissertation submitted by student William Peter Klein, and they evaluated his presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

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DEDICATION

To my parents
Daniel and Cindy Klein

That which does not kill us makes us stronger.
-Friedrich Nietzsche

If I have seen further than others,
It is by standing upon the shoulders of giants.
-Isaac Newton
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My journey at Boise State University began when I first met with Dr. Bill Knowlton in 2008 to express my interest in the Materials Science Graduate Program. Two years later, after I completed U.S. Army flight school, I returned for a second meeting. I met with current students and other faculty in the Nanoscale Materials and Device Research Group and was later accepted as a group member. After about six months, I began working with Dr. Wan Kuang, who became my primary advisor.

Wan’s guidance and mentorship over the past five years have undoubtedly positively influenced my life. Dr. Kuang has taught me countless scientific and life skills and has provided me with a computer science and programming background that has been extremely useful and has saved me time analyzing my data. I am truly grateful to have had such a multi-talented advisor.

During my time in this program, I have had the opportunity to travel to Washington D.C. and Munich, Germany. Most recently, in Washington D.C., I worked at the Naval Research Laboratory (NRL) with Dr. Igor Medintz and his postdoctoral researcher, Dr. Sebastian Diaz, for over five months. I would like to express my deep appreciation and gratitude to Dr. Medintz for providing me this opportunity, helping me clarify my future in defense-related research, and sparking
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ABSTRACT

Near-field energy transfer has great potential for use in nanoscale communications, biosensing, and light harvesting photonic devices. However, the light collecting and energy transferring efficiency of current devices is poor, resulting in few commercially available applications. Current human-made light harvesting devices lack the benefits of natural selection. Natural systems are typically highly optimized and highly efficient. For example, transfer efficiency in photosynthesis is greater than 90%.

In this work, two classes of optical devices were designed, synthesized, and characterized: Plasmonic waveguides and FRET-based photonic devices. In the case of plasmonic waveguides, a multi-scaffold DNA origami synthesis method was developed to fabricate linear waveguides with 10-nm diameter gold nanoparticles. Precise control over interparticle gaps and interchromophore distances was demonstrated. Using a similar approach, DNA labeled fluorophores were arranged in linear and branched geometries to form FRET-based photonic wires and light harvesting devices.

Recently, homogeneous FRET (homoFRET) has emerged as a potential way of increasing the transfer efficiency of photonic wires. However, little is known about the design principles needed to construct such devices. To address this knowledge
gap, linear photonic wires, and three light harvesting devices were designed, synthesized, and characterized. All the devices contained a homoFRET region to extend the energy transfer distance. Over 50 different FRET-based photonic wires with different homogeneous FRET configurations were evaluated. Several configurations were found that resulted in a higher end-to-end efficiency despite possessing fewer dyes. A six-fold antenna gain was achieved in the case of the light-harvesting devices. The findings demonstrate that homoFRET can be used to increase the energy harvesting capability of photonic devices. In general, the work also showed that DNA nanotechnology can be used to self-assemble a variety of photonic devices. Additionally, the work has established some basic design rules that will enable the bottom-up assembly of more elaborate devices.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AF488</td>
<td>Alexa Fluor 488 fluorophore</td>
</tr>
<tr>
<td>AF647</td>
<td>Alexa Fluor 647 fluorophore</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
</tr>
<tr>
<td>bp</td>
<td>Basepair</td>
</tr>
<tr>
<td>BSPP</td>
<td>Bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge-coupled device</td>
</tr>
<tr>
<td>Cy</td>
<td>Cyanine fluorophores</td>
</tr>
<tr>
<td>DF</td>
<td>Darkfield</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>2xD2</td>
<td>Double nanotube with dimer array (28 nm spacing)</td>
</tr>
<tr>
<td>dsDNA</td>
<td>Double stranded DNA</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>FRET</td>
<td>Förster Resonance Energy Transfer</td>
</tr>
<tr>
<td>Gbps</td>
<td>Gigabits per second</td>
</tr>
<tr>
<td>AuNP</td>
<td>Gold nanoparticle</td>
</tr>
<tr>
<td>heteroFRET</td>
<td>Heterogeneous Förster Resonance Energy Transfer</td>
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<tr>
<td>homoFRET</td>
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</tr>
<tr>
<td>keV</td>
<td>Kilo electron volt</td>
</tr>
<tr>
<td>LM</td>
<td>Longitudinal mode</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>Magnesium chloride</td>
</tr>
<tr>
<td>MHz</td>
<td>Megahertz</td>
</tr>
<tr>
<td>mM</td>
<td>Millimolar</td>
</tr>
<tr>
<td>Milli-Q</td>
<td>Millipore trademark name</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>nM</td>
<td>Nanomolar</td>
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<tr>
<td>ns</td>
<td>Nanosecond</td>
</tr>
<tr>
<td>2xD3</td>
<td>Nanotube nanorail with dimer array (28 nm spacing)</td>
</tr>
<tr>
<td>NRL</td>
<td>Naval Research Laboratory</td>
</tr>
<tr>
<td>nt</td>
<td>Nucleotide</td>
</tr>
<tr>
<td>Pbps</td>
<td>Petabit per second</td>
</tr>
<tr>
<td>PHz</td>
<td>Petahertz</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>pm</td>
<td>Picometer</td>
</tr>
<tr>
<td>ps</td>
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</tr>
<tr>
<td>rcf</td>
<td>Relative centrifugal force</td>
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<td>ssDNA</td>
<td>Single strand DNA</td>
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<tr>
<td>6HB</td>
<td>Six-helix bundle</td>
</tr>
<tr>
<td>SYBR</td>
<td>Synergy brands, Inc.</td>
</tr>
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<td>THz</td>
<td>Terahertz</td>
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xxxi
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<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>TCSPC</td>
<td>Time-correlated single photon counting</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TM</td>
<td>Transverse mode</td>
</tr>
<tr>
<td>TAE</td>
<td>Tris base, acetic acid and EDTA</td>
</tr>
<tr>
<td>TCEP</td>
<td>Tris(2-carboxyethyl)phosphine</td>
</tr>
<tr>
<td>TBE</td>
<td>Tris, borate, and EDTA</td>
</tr>
<tr>
<td>WTE</td>
<td>Wire transfer efficiency</td>
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</table>
LIST OF SYMBOLS

\( \sigma \) Absorption cross-section

\( AE \) Antenna effect

\( E_{ae} \) Anywhere-to-end efficiency

\( F_D \) Area of the donor fluorescence peak in the absence of an acceptor

\( F_{DA} \) Area of the donor fluorescence peak in the presence of an acceptor

\( \kappa \) Dipole orientation factor

\( I_D \) Donor emission spectrum normalized to unity

\( r_{DA} \) Donor-acceptor distance

\( E_w \) Efficiency white light

\( E_{ee} \) End-to-end efficiency

\( \sigma_{AD} \) Error in integrated fluorescence component area

\( \sigma_D \) Error in the integrated fluorescence component area for the donor

\( \sigma_{\Phi_A} \) Error in the quantum yield acceptor

\( \sigma_{\Phi_D} \) Error in the quantum yield donor

\( AE_E \) Experimental antenna effect

\( \epsilon \) Extinction coefficient

\( R_o \) Förster distance

\( I_{Cy5.5, 515 \text{ nm}} \) Fluorescence intensities of Cy5.5 (excitation of Cy3 donor at 515 nm)

\( I_{Cy5.5, 635 \text{ nm}} \) Fluorescence intensities of Cy5.5 (excitation of Cy3 donor at 635 nm)
<table>
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<td>$I_{Cy5.5, 685 \text{ nm}}$</td>
<td>Fluorescence intensities of Cy5.5 (excitation of Cy3 donor at 685 nm)</td>
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<td>$\tau$</td>
<td>Fluorescence lifetimes</td>
</tr>
<tr>
<td>$\phi_D$</td>
<td>Integrated component of the donor in the absence of any acceptor</td>
</tr>
<tr>
<td>$\phi_A$</td>
<td>Integrated component of the terminal acceptor</td>
</tr>
<tr>
<td>$\phi_{AD}$</td>
<td>Integrated component of the acceptor in the presence of a donor</td>
</tr>
<tr>
<td>$\phi_{Cy3.5\rightarrow Cy5.5}$</td>
<td>Integrated fluorescence area of Cy5.5 from the Cy3.5-Cy5.5 device</td>
</tr>
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<td>$\phi_{AF488}$</td>
<td>Integrated fluorescence of the donor in the absence of any acceptor</td>
</tr>
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<td>$\phi_{AF488}$</td>
<td>Integrated fluorescence of the AF488 donor alone</td>
</tr>
<tr>
<td>$\phi_{Cy5.5}$</td>
<td>Integrated fluorescence of Cy5.5 acceptor in the presence of all dyes</td>
</tr>
<tr>
<td>$N$</td>
<td>Number density</td>
</tr>
<tr>
<td>$J$</td>
<td>Overlap integral</td>
</tr>
<tr>
<td>$\Phi_D$</td>
<td>Quantum yield (QY) of the donor</td>
</tr>
<tr>
<td>$\Phi_A$</td>
<td>Quantum yield (QY) of the terminal acceptor</td>
</tr>
<tr>
<td>$\Phi_{AF488}$</td>
<td>Quantum yield AF488</td>
</tr>
<tr>
<td>$\Phi_{Cy5.5}$</td>
<td>Quantum yield Cy5.5</td>
</tr>
<tr>
<td>$Q_m$</td>
<td>Quantum yield of dye $m$</td>
</tr>
<tr>
<td>$n$</td>
<td>Refractive index</td>
</tr>
<tr>
<td>$p(k)$</td>
<td>The molar concentration of construct $k$</td>
</tr>
<tr>
<td>$S$</td>
<td>The number of different constructs in the ensemble</td>
</tr>
<tr>
<td>$M$</td>
<td>The number of different types of dyes</td>
</tr>
<tr>
<td>$N_k$</td>
<td>The number of dyes in the $k$th construct</td>
</tr>
<tr>
<td>$N^m_k$</td>
<td>The number of dyes of type $m$ in construct $k$</td>
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CHAPTER ONE: INTRODUCTION

Photonic devices generate, manipulate, or transfer photons. Photonic devices operate in the frequency range of 1 THz to 10 PHz, which corresponds to the wavelengths ranging from 300 $\mu$m to 30 nm. However, many photonic devices function in the relatively narrow visible wavelength region that ranges from 390 to 760 nm.\(^1\) Specific functions are engineered by changing the material composition of components and/or the geometric shape and configurations of the individual components. Metal nanoparticles, quantum dots, and fluorophores are examples of components that interact with light in unique ways. Over the last decade, a wide range of functional devices have been demonstrated, these include waveguides,\(^2\) beam splitters,\(^3\) phase shifters,\(^4\) and light harvesting devices.\(^5\)

Photonic devices offer many key advantages over current electronic devices. These include higher bandwidths, higher immunity to interference, and the absence of the Joule effect that leads to the generation of unwanted thermal heat.\(^6\) Companies such as Intel have recognized the potential for such devices and are actively investing in photonic-related research and development. For example, Intel currently produces data interconnects that operate at speeds of up to 100 gigabits per second (Gbps), or $1.0 \times 10^{11}$ bits per second.\(^7\) However, theoretical limits for optical fiber communications are estimated to be as high as 1 petabit per second (Pbps), or $10^{15}$
bits per second. The significant discrepancy in transfer rates exists in part due to the bottleneck that occurs when converting optical signals to an electrical signal. Photonic devices offer the potential to eliminate this bottleneck by eliminating the need for the electrical signal conversion since all the electrical components could be replaced with their optical equivalent devices.

Integrated optical devices are typically produced using top-down photolithography to achieve the precise nanometer dimensional specifications required. The top-down process, while well-suited for creating specific dimensions, requires specialized and expensive photolithography equipment that is cost prohibitive for most researchers.\(^8\) An alternative approach is to construct photonic devices using molecular self-assembly.\(^9,10\) Molecular self-assembly is the arrangement of molecules using non-covenant interactions such as hydrogen bonding and van der Waals interactions.\(^11\) An example of molecular self-assembly is DNA self-assembly which uses complementary Watson-Crick base-pairing to fold DNA into arbitrary three-dimensional shapes.\(^12\) DNA nanotechnology was pioneered by Dr. Nadrian Seeman as a method of crystallizing proteins.\(^13\) It was not until the technique was later refined by Hao Yan in 2003 and Paul Rothemund in 2006, both of whom replaced the relatively short DNA scaffold strand with a DNA plasmid, that the method rapidly grew.\(^14,15\) Bacteriophage M13mp18, containing 7,249 nucleotides, was used in the latter case. DNA origami has been used for the precise attachment and positional control of functionalized elements such as gold nanoparticles (AuNPs) quantum dots, and/or fluorophores.\(^16,17\) The attachment precision is better than 2 nm. The method also has a high level of specificity resulting
from the myriad of DNA sequence combinations. The specificity of the approach makes DNA nanotechnology useful for site-specific attachment of many different functional components.\textsuperscript{18,19}

Figure 1.1. DNA origami production workflow. The process begins with design and modeling. The second and third steps involve joining and folding the staple and scaffold strands. Excess staple strands are then removed. Finally, the folded origami can be characterized or functionalized. An atomic force microscopy (AFM) scan of a ring origami on the surface of mica is shown in step 5. 10 nm AuNPs attached to specific ring locations are shown in the lower right of the figure as an example of the types of site-specific nanoscale modifications that are possible.

The typical procedure for designing, synthesizing, and characterizing templated photonic devices is shown in Figure 1.1. The desired geometry is designed using open-source software such as Cadnano.\textsuperscript{20} The 3D structure and mechanical rigidity of the origami are modeled using CanDo.\textsuperscript{21} The goal of modeling is to optimize the scaffold and staple routing of the origami maximize formation yield.
The number of crossovers, placement of crossovers, the length of base pair hybridization regions, and the specific DNA sequences effect the thermal and mechanical stability of the DNA origami. Once the scaffold and staple routing is optimized and the design is final DNA sequences are commercially synthesized. The sequences for the staples are complementary to specific locations on the scaffold.

Next, the staples are mixed with the scaffold in a buffer solution and then annealed at an elevated temperature. DNA folding is driven by the formation of complementary base pairs, which lower they free energy of the system. The geometric shape takes form as the staple strands begin to bind to specific domains of the scaffold. Salt is added in the form of magnesium ions to screen electrical static interactions. Excess staple strands are added to increase the probability of complete nanostructure formation and later removed. Functionalized elements such AuNPs where then attached at specific binding sites on the nanostructure. The binding sites were created by extending certain staples strands with sequences that are complementary to the functionalized elements. The locations of the binding sites are carefully selected so that the functional elements, such as AuNPs bind in the desired geometry required to create the designed photonic device.

The nanoscale dimensions of DNA-templated photonic devices are similar to their electronic counterparts. For example, Intel’s latest transistor has dimensions of 42x70 nm (based on 14 nm lithography), which is comparable to a 10 nm AuNP beam splitter.22 These dimensions are much smaller than the wavelength of visible light. However, in a photonic waveguide, light can be transmitted at a reduced wavelength when confined inside the waveguide. As the light enters the waveguide, the
electromagnetic waves’ optical modes are converted into non-radiating surface plasmons.\textsuperscript{23} The surface plasmons have wavelengths on the order of tens of nanometers compared to hundreds of nanometers in free space.

Surface plasmon resonance occurs when the conduction electrons are stimulated by light with a frequency close to the natural frequency of the conduction electrons in the material.\textsuperscript{24} The plasmon frequency depends on the size and shape of the metal particles.\textsuperscript{25} The basic concept of surface plasmon resonance is depicted in Figure 1.2. When the conduction electrons oscillate at the same frequency of the light waves’ electric field, they create a plasmonic wave. For an excellent and complete review on AuNP surface plasmons’ resonance, the author recommends S. Ghosh’s and T. Pal’s relevant article.\textsuperscript{26}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Schematic depicting how the conduction electrons in AuNPs oscillate when excited with an incoming light wave, leading to surface plasmon resonance.}
\end{figure}

Gold nanoparticles (AuNPs) can be arranged into a variety of geometries to create photonic devices that can fulfill functions as filters, directional couplers, beam splitters, phase shifters, etc.\textsuperscript{27,28} Precise control over nanoparticle size, spacing, and spatial arrangement offers the potential for a complete set of nanoscale optical components. Waveguides are photonic devices that use near-field physical
phenomena to transfer light. Varying the nanoparticle diameter, as well as the distance between adjacent nanoparticles controls the near-field coupling between particles.\textsuperscript{29} Chapter Two of this dissertation describes research in this direction. More specifically, the design, synthesis, and characterization of self-assembled plasmonic waveguides created by arranging AuNPs onto a multi-scaffold DNA origami nanostructure.\textsuperscript{30} In this work, AuNPs are arranged in sets of dimers and trimers on DNA templates to create waveguides. Several different DNA template waveguide designs were optically characterized and found their optical properties to be in agreement with numeric simulations. Additionally, the waveguides’ structure was directly correlated to the optical properties. Results also demonstrated that attaching AuNPs to the cross-linked six-helix bundle nanotube template was the best method of producing linear waveguides.

Light-harvesting is another branch of current nanophotonics research. One of the goals of this research is to build a synthetic light-harvesting device that rivals the light collecting and transfer efficiency achieved in natural photosynthesis. Natural photosynthesis utilizes specific geometric arrangements of green chlorophyll pigments that act like fluorophores to funnel energy to specific reaction sites.\textsuperscript{31} The process is highly efficient and is made possible in part by the particular arrangement of the pigments.\textsuperscript{32} The pigment molecules in plants have been shown to act as antenna complexes that increase the light-collecting efficiency.\textsuperscript{33} The energy transfer mechanism in natural photosynthesis is Förster Resonance Energy Transfer (FRET). Thus, it should be possible to mimic light-harvesting by arranging fluorophores in specific geometries on DNA-templates with the goal of creating a photonic device.

Over the last few years, researchers have demonstrated the successful construction of many nano-sized devices that use FRET, ranging from biosensors to
energy-harvesting devices. A schematic of the FRET process is shown in Figure 1.3. Incoming light excites an electron to a higher energy state, as shown in the lower part of the figure. The exciton then relaxes through an internal conversion process. Once realized, the exciton couples to the acceptor’s energy state, transferring energy to the acceptor. The exciton relaxes a second time and finally emits a photon of lower energy than the initial excitation. The process is commonly depicted using a Jabłoński diagram (named after Aleksander Jabłoński, who developed it as a way of explaining the physics of fluorescence in his 1933 paper). FRET is efficient at moving excitons at nanometer scales but is limited at longer distances because the rate of energy transfer is dependent on the sixth power of the distance between the donor and acceptor.

**Förster Resonance Energy Transfer**

![Diagram of Förster Resonance Energy Transfer](image)

**Figure 1.3.** Schematic depicting FRET between a pair of fluorophores.

There are two types of FRET: heterogeneous FRET (heteroFRET) and homogeneous FRET (homoFRET). HeteroFRET is the transfer between two different
fluorophores, whereas homoFRET is the transfer between two identical fluorophores. At first, this distinction might seem trivial. However, it is critical because different transfer laws govern their performance. For example, heteroFRET is an energy cascade with downhill directionality as opposed to homoFRET in which the directionality is lost. In homoFRET, two or more molecules act as both the donor and the acceptor. Their energy levels, in theory, are identical and thus provide pathway for a lossless energy transfer. However, the random walk nature of homoFRET sets a limit on the overall transfer distance. In plants, up to 300 individual pigments form a sphere around the reaction center transfer sunlight and achieve high light-harvesting efficiencies. Thus, a better understanding of the design principles would allow for the creation of higher efficiency light-harvesting devices that may provide alternative methods for improving solar energy collection. While much is known about the physics governing simple FRET systems that consist of only a few fluorophores, much less is known about systems comprised of many dyes, which are known as FRET networks. Understanding engineering design parameters such as the dye spacing or Förster distance, \( R_0 \), the particular donor and acceptor pairs, and the dye’s spatial configurations will help advance the field.

Chapter Three describes designing, constructing, and characterizing a 30 nm long DNA origami FRET wire consisting of 14 fluorophores. The DNA template for the wire consists of six DNA helices in the same hexagonal arrangement of the six-helix bundle nanotube used to create the waveguides described in Chapter Two. The template was approximately 30 nm in length. The fluorophores were attached to the center of the nanotube. In this investigation, the steady-state and time-resolved
fluorescence spectroscopy was used to characterize over fifty photonic wires each with different homoFRET configurations. The end-to-end efficiency and wire transfer efficiency was determined for each photonic wire. Many wire configurations were found to exhibit nearly equivalent transfer efficiencies. However, several configurations were found to exhibit relatively high efficiencies. A nearly 200% increase in the end-to-end efficiency was observed when the first fluorophore was removed from the wire compared to the full wire.

Work related to understanding complex networks consisting of heteroFRET and homoFRET are covered in Chapter Four of this dissertation. In this work, the light-harvesting and end-to-end energy transfer efficiencies of three photonic devices were evaluated. The photonic devices were created by arranging fluorophores on DNA templates. Each DNA template was capable of six possible homoFRET configurations, allowing for the number of Cy3 homoFRET repeats on each arm of the device to be increased from one to six. The increasing number of homoFRET repeats was found to increase the antenna gain up to five repeats, after which it began to decrease. The transfer efficiency of these photonic devices, while impressive, were still considerably lower than natural systems. Our devices did demonstrate a relatively high light-collecting efficiency or antenna gain, indicating that our design was a step in the right direction.
REFERENCES


CHAPTER TWO: MULTI-SCAFFOLDED DNA ORIGAMI WAVEGUIDES

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Multiscaffold DNA Origami Nanoparticle Waveguides

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2.1 Abstract

DNA origami templated self-assembly has shown its potential in creating rationally designed nanophotonic devices in a parallel and repeatable manner. In this investigation, we employ a multi-scaffold DNA origami approach to fabricate linear waveguides of 10-nm diameter gold nanoparticles. This approach provides independent control over nanoparticle separation and spatial arrangement. The waveguides were characterized using AFM and far-field polarization spectroscopy. This work provides a path towards large-scale plasmonic circuitry.

2.2 Introduction

Driven by the promise of providing a relatively economical and massively parallel way of fabricating complex nano-structures, interest in DNA-directed self-assembly continues to grow.1–4 By taking advantage of the specific binding between complementary DNA sequences, oligonucleotides can be formed into a variety of rationally designed shapes through a variety of processes including DNA origami,5 molecular canvas,6 DNA gridiron,7 and designs that incorporate multiple scaffolds.8 A primary driver of forming such nanoscale structures is the precise assembly of nanoparticles into well controlled geometries in order to achieve novel material properties based on the collective behavior of the assembly.9,10 For example, chains of closely spaced metal nanoparticles can guide electromagnetic energy below the diffraction limit by converting optical modes into non-radiating surface plasmons.11–15 Waveguiding is made possible due to the resonant coupling between nanoparticles, and the resonant coupling frequency can be controlled by varying the
nanoparticle diameter and the distance between adjacent nanoparticles.\textsuperscript{16–18} Metal nanoparticles can also be arranged into a variety of geometries that can fulfill functions such as filters, directional couplers, beam splitters, and phase shifters.\textsuperscript{19,20} Thus, precise control over nanoparticle size, spacing, and spatial arrangement offers the potential for creating a complete set of sub-diffraction nanoscale optical components.

To enable efficient nanoparticle-based waveguiding, the plasmon modes of adjacent nanoparticles must be strongly coupled, which requires an inter-particle gap smaller than the radii of the particles.\textsuperscript{21,22} Previously, self-assembled plasmonic waveguides have been fabricated using meniscus force deposition\textsuperscript{17,23} and direct DNA-based coupling.\textsuperscript{10,24,25} These techniques allow the spacing between nanoparticles to be carefully controlled, enabling strong plasmon coupling, but they offer little control over the spatial arrangement. For instance, incorporation of multiple periodicities within a linear nanoparticle array would be extremely difficult, yet the arrangement of gold nanoparticles (AuNPs) into superlattices has been shown to allow precise engineering of waveguide mode dispersion.\textsuperscript{26,27} A directed self-assembly method, such as DNA origami, has been shown to offer control over both inter-particle gap and spatial arrangement,\textsuperscript{28–30} however long linear super-lattices incorporating multiple scaffolds have not yet been demonstrated.

Here, we report the directed self-assembly of AuNPs into linear semi-rigid superlattice arrays using single and multi-scaffold DNA origami nanotubes. We demonstrate high-yield synthesis and high-fidelity to the designed target structure. Our design achieved a 14 nm center-to-center spacing between adjacent 10 nm
diameter AuNPs for visible spectrum sub-diffraction plasmonic waveguiding. Structural rigidity and minimal defects are critical factors for successful waveguide fabrication, and both were achieved by cross-linking origami nanotubes into multi-scaffold templates. Individual characterization of superlattice plasmonic waveguides revealed strong surface plasmon coupling in good agreement with simulations.

2.2.1 Design

Plasmonic superlattice waveguides were formed by self-assembling AuNPs into linear arrays using six-helix DNA origami nanotubes with a designed diameter of 6 nm and length of 412 nm. Figure 2.1 depicts the five plasmonic waveguide structures that were designed, synthesized, and characterized in this study. The 1xD1, 1xD2, and 1xT waveguide designs employ a single six-helix nanotube, as shown in Figure 2.1(a-c), while the 2xD2 and 2xD3 designs employ two origami nanotubes, as shown in Figure 2.1(g-h). To clarify, “1x” and “2x” describe the number of nanotubes involved in each design, while “D” and “T” indicate that the waveguide consisted of superlattice arrays of AuNP dimers and trimers, respectively. 1xD1 and 1xD2 waveguides consisted of AuNP dimer sets periodically arranged at 70 nm and 28 nm spacings, respectively. 1xT consisted of AuNP trimer sets spaced by 56 nm. In all designs, the nanoparticle binding sites were separated by 14 nm within a dimer or trimer set.

Nanoparticle binding sites consisted of two identical 15 nucleotide (nt) sequences that extended from specific staple strands distributed along the nanotube
Single Nanotube Designs

a) 1xD1
14 nm 70 nm

DNA Nanotube

b) 1xD2
28 nm


c) 1xT
56 nm

Double Nanotube Designs

g) 2xD2
28 nm

h) 2xD3
Cross-link 28 nm
Nanorail

Cross-linking
Tube 1 Tube 2
axis. To prevent a single nanoparticle from hybridizing to two adjacent binding sites, adjacent binding sites were designed with two unique sequences, denoted “A” and “B” in Figure 2.1. By modifying the location of the “A” and “B” binding sites on the nanotube, three different waveguides were synthesized. 1xD1 and 1xD2 waveguides each consisted of a periodic “AB” pattern, while 1xT waveguides consisted of a periodic “ABA” pattern. 10 nm diameter AuNPs were conjugated with thiolated single-stranded DNA (ssDNA) sequences complementary to “A” and “B” to enable site specific hybridization to the nanotubes. The formation of 1xT waveguides required the hybridization of “B” AuNPs to the nanotube to occur before that of “A” AuNPs. This sequential hybridization procedure promotes steric hindrance and prevents “A” AuNPs from bridging two “A” sites over a “B” binding site. Design schematics, nanotube sequences, synthesis protocols, the AuNP conjugation process, and AuNP attachment yield are described in Section 2.6.1.

Figure 2.1 (preceding page). (a-c) Schematics of the three single nanotube waveguides with 70, 28, and 56 nm periodicity between AuNP sets. The “A” and “B” labels on the individual AuNPs refer to the DNA attachment sequence used to functionalize the AuNPs. (d-f) Negatively stained bright field TEM images of the waveguides for each corresponding design. (g-h) The double nanotube designs each consisted of two nanotubes to increase mechanically rigidity and the number of DNA sticky-ends that bind each AuNP to the tube. The 2xD2 design featured single 1x2D2 nanotubes cross-linked through AuNPs, while the 2xD3 design incorporated 18 cross-linking ssDNA strands at nine locations, equally spaced along the waveguides. All designs incorporated a 14 nm center-to-center spacing between adjacent 10 nm diameter AuNPs. TEM images for double nanotube waveguides are shown in (i) and (j). A cross-section highlighting the routing of two cross-linking strands shown in yellow and purple. The blue and orange cylinders represent the DNA double helix. The staple strands have be omitted.
Negatively stained transmission electron microscope (TEM) images of successfully synthesized structures are shown to the right of each design schematic in Figure 2.1. As can be seen from the images, each target design was successfully synthesized, confirming the power of DNA-directed self-assembly in controlling both nanoparticle spacing and spatial arrangement. The TEM sample preparation is described in Section 2.6.2. Despite a high nanoparticle attachment yield, generally above 90 percent, characterization of the 1xD1, 1xD2 and 1xT waveguides revealed that AuNPs could fall on either side of the nanotube when depositing them on a substrate. This deviation from linearity was sufficient to cause the polarization dependence of the waveguides’ scattering spectra to be poorly defined, as discussed below. Several examples are shown in Section 2.6.3. To better control the orientation and location of the AuNPs and to increase the mechanical rigidity of the waveguides, plasmonic waveguide arrays assembled on two DNA origami nanotubes were developed.

The 2xD2 waveguide arrays consist of two parallel nanotubes bound together by AuNPs as shown in Figure 2.1g. The structure resembles a ladder with AuNPs as the rungs of the ladder and the nanotubes as the legs. The structure was synthesized in the same manner as the 1xD2 structure; however, a modified AuNP to nanotube binding site concentration ratio was utilized. The AuNP to nanotube binding site ratios were 5:1 and 2:1 for the 1xD2 and 2xD2, respectively. Lowering the AuNP concentration promoted the formation of a laddered structures in which AuNPs were shared between two nanotubes. This approach added an additional constraint on AuNP placement and resulted in much higher nanoparticle linearity. However, the
repeating binding sites on each tube led to the formation of waveguides with longitudinal misalignment between independent nanotubes, as shown in Section 2.6.4. The yield of well-formed waveguides was low using this approach.

An alternative multi-scaffold approach is to intentionally construct nanoparticle templates by cross-linking two complementary nanotubes, as illustrated by the 2xD3 design in Figure 2.1h. Two nanotubes, designated tube 1 and tube 2, were designed to cross-link by modifying 18 of the original staple strands. An enlarged cross-section of the 2xD3 waveguide is shown, and depicts the routing of the cross-linking strands shown in yellow and purple. The cylinders in the figure represent the double helix formed by the scaffolds and staple strands of the self-assembled waveguide. Staple strands and scaffold strands are not shown for clarity. Nine of the original 42 nt staple strands from one tube were lengthened by 14 nt to cross-link to the complementary nanotube, which has nine staple strands shortened by 14 nt. The same scheme was applied also in reverse to form a total of nine double inter-tube cross-links. Tubes 1 and 2 were synthesized and filtered separately and then hybridized to each other to form a single cross-linked structure, designated as 2xD3 and referred to as the “nanorail”. The formation of the nanorail effectively doubled the number of sticky-ends per binding site to four, which improved the yield of well-formed waveguides in addition to eliminating the longitudinal misalignment between independent nanotubes that was observed with the 2xD2 waveguides. As can be seen in Figure 2.1j, the synthesized 2xD3 structures exhibited an high AuNP attachment yield and high nanoparticle linearity.
Once the yield of well formed structures was sufficiently high, greater than 90%, optical characterization of the waveguides was performed to investigate how different spatial arrangements of AuNPs affected the surface plasmon resonance in each waveguide design. To prepare the self-assembled waveguides for topographical and optical characterization, the self-assembled waveguides were deposited onto atomically flat mica disks that were previously glued to a glass slide with optical epoxy as outlined in Section 2.6.5. To increase the scattering cross section and decrease the gap between pairs of the AuNPs, the AuNPs in some samples were enhanced using electroless deposition, as described in Section 2.6.6. All samples were completely dried with nitrogen gas prior to performing the AFM and darkfield microscopy characterizations.

2.3 Results and Discussion

During the AFM and darkfield characterization, low surface concentrations of \( \sim 10 \) waveguides per \( 30 \times 30 \ \mu m^2 \) were found to be the most desirable for registration of individual waveguides. This low concentration greatly reduced excess scattering of light by neighboring waveguides. AFM characterization was performed in non-contact tapping mode using a Bruker Icon AFM equipped with a Bruker Fast-Scan head. The AFM scanning techniques are detailed in Section 2.6.7. During AFM characterization, four high-resolution \( 20 \times 20 \ \mu m^2 \) non-contact mode height images were recorded in succession such that the images had approximately 10 \( \mu m \) of overlap to account for the thermal drift and moving the mechanical stage below its limit of resolution. The four images were post processed using Nanoscope Analysis
(Bruker), WSxM, and ImageJ and then digitally combined into a single image covering approximately $30 \times 30 \, \mu m^2$. The independent AFM images were overlapped primarily using the unique pattern the individual waveguides created on the mica surface. Optical characterization of the waveguides was conducted using a spectrographic inverted darkfield microscope described in Supporting Information S8. By registration with fiducial reference marks, high-resolution AFM images and far-field optical spectra were collected from individual waveguides.

Figure 2.2a shows the schematic of a 1xD1 waveguide assembled on a single six-helix bundle DNA origami nanotube. An AFM image overlaid with a transparent darkfield image is shown in Figure 2.2b. The two white halos surrounding the waveguides originate from the darkfield image and are the result of the scattering of light. The black dots located in the centers of the halos are the individual waveguides imaged by AFM. The magnified AFM image of the selected waveguide is shown in the inset of Figure 2.2b. The scattering spectrum of the waveguide under white light illumination was measured and calculated, as shown in Figure 2.2c and 2.2d, respectively. Additional measured scattering spectra can be found in Section 2.6.8. The numerical calculation was performed with a finite element method using COMSOL, assuming a nanoparticle radius of 6.3 nm, determined as the average particle radius from the AFM scan of the waveguide and a center-to-center spacing of 14 nm. The simulations assumed a constant value of 1.56 for the refractive index of mica for all calculations presented in this letter. The effective dielectric constant of Au is dispersive in the visible wavelength and was taken from Christy and Johnson. The deviations observed in the measured scattering spectra from the calculated
spectra are attributed to the difference in the local index of refraction of mica which varies depending on crystallographic orientation. The measured spectra were fit to a set of calculations, each of which assumed a particular value for the refractive index of mica. It was determined that the refractive index of mica to accurately model the measured spectra was in the range of 1.56 to 1.60, consistent with the index of refraction supplied by the manufacturer.\textsuperscript{37} The refractive index of the DNA nanotubes was not considered in the simulations due to the fact that dsDNA has been shown to have a refractive index of 1.54 which is close to that of mica.\textsuperscript{38}
Figure 2.2. Plasmonic waveguide arrays assembled on a single six-helix bundle DNA origami nanotubes. The figure is divided into three columns, one for each of the waveguide array designs. The schematics for each design are shown at the top of each corresponding column. The combined AFM and darkfield images of the waveguides are shown in, (b), (f), and (j), with the inset of each image containing magnified AFM scans of the characterized waveguides. The bright halos surrounding the waveguides originate from the optical image and result from optical diffraction. The measured scattering spectra, (c), (g), and (k), and the calculated scattering spectra, (d), (h), and (i), of the waveguides are shown in the bottom two rows of the corresponding columns. The calculated spectra for each waveguide was determined through numerical modeling in COMSOL. The red-shifts between the measured and calculated spectra are attributed to variations in the local refractive index of mica used in the experiments.

The spacing between the AuNP dimer sets in 1xD2 waveguides (middle column of Figure 2.2) was 28 nm, as shown in the schematic. Although the waveguide shown in the inset of Figure 2.2f was fairly linear, few waveguides of this design possessed the linearity of this particular structure. The lack of linearity of the waveguides was identified as a common issue for waveguides assembled with a
single DNA origami nanotube. In addition, the deviations of scattering spectra among individual waveguides are significant, as is shown in Section 2.6.9.

Figure 2.2(i-l) shows the results for waveguides consisting of AuNP trimers. A noticeable redshift was observed by comparing the scattering spectra of 1xT waveguides with 1xD waveguides. This result indicates that the trimer waveguides have a stronger longitudinal mode (LM) than the dimer waveguides, as expected. Additional spectra of 1xT waveguides can be found in Section 2.6.9. The spectrum calculated using a AuNP radius of 6.2 nm, determined as the average particle radius from the AFM scan of the waveguide, a mica refractive index of 1.56, and AuNP spacing of 14 nm for the 1xD waveguide is shown in Figure 2.2l.

The strong agreement between the measured and calculated spectra support the ability of DNA-directed self-assembly to form spatially complex superlattice arrangements of nanoparticles. As an additional assessment of the fidelity of DNA-directed self-assembly, the inter-particle spacing of the single nanotube waveguide arrays was quantitatively determined by averaging the distance between adjacent nanoparticles contained within the dimer and trimer sets. In each design, the inter-particle spacing within a dimer or trimer set was designed to be 14 nm. The measured inter-particle spacing of the single nanotube waveguides were found to be 14 nm (n=101), 16 nm (n=62), and 13 nm (n=100), for the 1xD1, 1xD2, and 1xT waveguides, respectively. All measurements had a standard deviation of 2 nm, which is close to the previously reported value of 1.4 nm for similar structures. These results further support that DNA-directed self-assembly is capable of controlling nanoparticle spacing, and thus inter-particle gap.
Despite the control of AuNPs within dimer and trimer sets, in general, waveguides assembled on single DNA origami nanotubes were found to lack the mechanical rigidity required for the formation of highly linear waveguide arrays. By comparing calculated and measured scattering spectra, it was determined that the measured spectra were produced by LM and transverse mode (TM) plasmon resonances of the AuNP dimers. Yet, the scattering spectra for both the 1xD1 and 1xD2 waveguides showed weak polarization dependency, as shown in Section 2.6.9. The deficiency of a well-defined polarization dependency of the scattering spectra is primarily attributed to the non-linearity of the dimer and trimer sets on the waveguides. Furthermore, AuNPs would bind and lay on either side of the nanotube further compromising the optical properties of the waveguides, as previously described. These factors indicate that single DNA origami nanotubes are not suitable templates for fabricating linear plasmonic waveguides.

The linearity of the waveguides was greatly improved with the addition of a second nanotube orientated along the long axis of the structure, as illustrated by the 2xD2 and 2xD3 designs shown in Figure 2.3a and 2.3f. The 2xD2 waveguides consisted of two nanotubes linked by AuNP bridges, while the 2xD3 waveguides were cross-linked with ssDNA strands. Figure 2.3(b-d) shows AFM and optical results for one 2xD2 waveguide. The greatly improved alignment of the dimers due to the second nanotube resulted in well defined polarized scattering spectra as shown in Figure 2.3d. The calculated polarized far-field spectra are shown in Figure 2.3e, which assumes a AuNP radius of 6.0 nm and a center-to-center distance of 14 nm. The spectral spacing between the TM and LM modes of the collected polarized spectra is in good agreement with the calculated values. Additional scattering spectra can be found in Section 2.6.9.
Figure 2.3. Plasmonic waveguide arrays assembled on two DNA origami nanotubes. (a-e) Show results for the 2xD2 design and (f-j) show results for the 2xD3 design. The combined height AFM, darkfield, and magnified AFM scan of the characterized waveguides images of the waveguides are shown in (b) and (g). The measured scattering spectra of the waveguides shown in the AFM inset are shown in (c) and (h), and the polarized spectra are shown in (d) and (i). The calculated spectra of both LM and TM modes of the waveguides are shown as red squares and blue circles, respectively in (e) and (j). The LM and TM modes are collected with the polarizer parallel and perpendicular to the long axis of the waveguides, respectively. The spacings between the TM and LM modes of the collected polarized spectra agree with with the theoretical spacings.
In general, the laddered waveguides possessed the mechanical rigidity and linearity required for the formation of desired linear waveguides. However, the yield of well-formed waveguides was difficult to control. The periodic arrangement of identical binding sites on the nanotubes led to the hybridization of AuNPs to non-equivalent sites of the two nanotubes, resulting in a longitudinal misalignment between independent nanotubes, as previously discussed. The low-yield problem of the 2xD2 waveguide was corrected by designing two complementary nanotubes that could hybridize to form the template for the 2xD3 waveguides.

2.3.1 TEM and AFM Characterization

TEM and AFM characterization of 2xD3 waveguide arrays confirmed a much greater yield over 90% of well-formed, linear waveguide structures. The results for the 2xD3 design are shown in Figure 2.3(g-i). Complete AuNP attachment was observed and attributed to the two-fold increase in the number of AuNP binding tethers (four tethers per binding site) that were incorporated into the structure. The combined AFM and darkfield image is shown in Figure 2.3g, with a magnified view of the selected waveguide shown in the inset. The dimer alignment was greatly improved compared with waveguides assembled on a single origami nanotube. As a result, well defined polarization dependent scattering spectra were obtained, as shown in Figure 2.3i. Additional spectral measurements of the 2xD3 waveguide arrays are shown in Figure 2.16. The spectral spacing of 20 nm for the 2xD3 waveguides between the TM and LM modes of the collected polarized spectra agrees well with the calculated values. A radius of 5.4 nm and a center-to-center distance of
14 nm was used in the calculation in the spectra shown in Figure 2.3j. By comparing the scattering spectra from multiple 2xD3 waveguide measurements, as shown in Figure 2.16, excellent conformance was observed, indicating a high fidelity to the waveguide design. Additional agreement to the designed structure was obtained from measurements of the inter-particle spacing of the double nanotube waveguides. Spacings were found to be $14 \pm 2$ nm ($n = 103$) and $13 \pm 2$ nm ($n = 102$), for the 2xD2 and 2xD3 waveguides, respectively, in perfect agreement with the designs.

2.3.2 Waveguide Linearity

In order to quantitatively analyze the linearity of the synthesized waveguides, AFM scans were analyzed for each waveguide design. Linearity was characterized by measuring the length of a line constructed tangent to the end of a waveguide and extending to the point where the curvature of the waveguide deviated from the center of the drawn line, as is shown in Section 2.6.15. Lines were constructed beginning from each end of the waveguide, and the two lengths were averaged to give the average linearity length for each waveguide. The 1xD1 waveguide had an average linearity length of $168 \pm 78$ nm ($n = 47$). Compared to an average linearity length of $257 \pm 103$ nm ($n = 51$) for the 2xD3 waveguide. Individual waveguides were declared linear if their average linear length exceeded 95% of the designed 412 nm length. The fraction of linear nanotubes was determined by summing the total number of linear waveguides by the total number of waveguides analyzed. The 1xD1 and 2xD3 waveguides’ percent of linear waveguides were determined to be 41 and 63%, respectively. Use of three or four cross-linked nanotubes in a single waveguide are expected to increase the waveguide linearities further.
2.3.3 Refractive Index Effect

As a final comment, we note that the peak resonance wavelength of a waveguide strongly depends on the refractive index of its local environment. The calculated spectra are generally shifted 20 nm from those obtained by measurement, however the actual amount of wavelength shift depends on the actual index of refraction of the mica substrate below the waveguide. Red shifts of up to 40 nm depending on the surface area of the nanoparticles have been shown in simulations. The spectral differences can also be attributed to the size distribution of AuNPs. The calculated far-field scattering spectra assume all the AuNPs have the same radius and are perfectly spherical. In reality, the AuNPs attached to the waveguide have a distribution of sizes and are not perfectly spherical. The AuNPs used in this study had a standard deviation in diameter of 1 nm and were >95% spherical. The reduced sphericity causes a slightly enhanced resonance along the long axis of the particle. This deviation in size distribution has been shown to cause broadening of both the TM and LM peaks and leads to red shifts of both TM and LM modes. The calculated scattering spectra also assumed all the dimers to be in a completely linear configuration. Deviations in linearity of the waveguide would blue shift both the TM and LM peaks.

2.4 Conclusion

In summary, through DNA-directed self-assembly, we constructed linear plasmonic superlattice waveguide arrays with precise control of both inter-particle gap and spatial arrangement, exhibiting well-defined optical properties in agreement
with calculations. By conducting AFM and darkfield characterization on the same individual waveguides, we directly correlated structure and optical properties to show that the deviations of the orientations of AuNP dimer and trimer sets within the superlattice arrays have a significant impact on the polarization dependent scattering spectra. The use of a multi-scaffold, two-nanotube nanorail structure greatly improved the mechanical rigidity, and thus linearity, of the waveguides. These results indicate that with cross-linking of multiple DNA scaffolds, DNA origami can be used to fabricate relatively large and complex waveguiding structures.

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2.6 Supporting Information

2.6.1 Single Nanotube Designs

All of the superlattice array waveguide designs were based on a six-helix DNA origami nanotube described elsewhere. The M13mp18 scaffold layout is shown in Figure 2.4a. The scaffold nucleotide position is indicated at each crossover location. The staple strands are arranged into 86 columns and numbered from left to right. A segment of the total nanotube is shown in Figure 2.4b.

The 1xD1, 1xD2, and 1xT waveguide arrays each consist of single DNA origami nanotubes containing 172 DNA staple strands. The 1xD1 waveguide arrays were designed to have five AuNP dimers spaced at 70 nm along the nanotube, while the 1xD2 waveguides were designed to have ten individual AuNP dimers spaced at 28 nm along the nanotube. The 1xT waveguide was designed to have five sets of AuNP trimers spaced at 56 nm along the nanotube. A segment of the 1xD2 waveguide design is shown in Figure 2.4c. To create sets of independent nanoparticle binding sites, 15 nt long ”A” or ”B” sticky-ends were added to the 3’ ends of specific staple strands. As can be seen in Figure 2.4c, each binding site consists of two sticky-ends of the same sequence separated by 14 nt along the nanotube axis and located on adjacent helicies.

2.6.1.1 Double Nanotube Designs

The 2xD2 waveguide contained two 1xD2 waveguides laddered by AuNPs, and thus the sequences were the same for both waveguides. The 2xD3 waveguide contains two different six-helix bundle nanotubes that were synthesized separately.
and were designed to have a total of 18 cross-linking strands at nine locations along the two nanotubes. Two-dimensional segment of the 2xD3 waveguide is shown in Figure 2.4d. The cross-linking strands are shown in red and green. A two-dimensional and a three-dimensional cross-section of the 2xD3 waveguide is shown in Figure 2.4e and 2.4f. The design required the modification of 36 staples from the original six-helix nanotube. The waveguide was designed to have ten individual AuNP dimers spaced at 28 nm along the longitudinal axis. The cross-links from tube 1 to tube 2 were placed starting at column four on tube 1 with a nine column (126 bases) periodicity to column 76. The cross-links from tube 2 to tube 1 were placed starting at column five on tube 2 with a nine column periodicity to column 77. This design effectively doubled the number of AuNP tethers per binding site from two to four.

Figure 2.4 (preceding page). Schematics of the scaffold and staple strands of the DNA origami waveguide arrays. (a) A two-dimensional layout of the scaffold strand in the original six-helix bundle nanotube. The numbers represent the conventional nucleotide number of M13mp18 at each crossover. (b) The first 14 columns of the original six-helix bundle nanotube. The staple motif is shown extending from column 3 to column 5. (c) A segment of the 1xD2 waveguide staple layout extending from column 8 to column 22. The "A" and "B" represent the staples that were extended with sticky-ends that were complementary to the conjugated AuNPs. The extended staples are shown in purple and green for the "A" and "B" sticky-end sequences, respectively. (d) A segment of the 2xD3 waveguide displaying how the two nanotubes are cross-linked to each other at helix 4 on tube 1 to helix 1 on tube 2. The cross-linking strands are shown in dark green and dark red. (e) Two-dimensional schematics showing the routing of the cross-linking ssDNA strands. The blue circles represent the DNA scaffold and the red and green arrows represent the cross-linking staple strands. (f) Cross-sectional view of the 2xD3 structure highlighting the crossover strands, shown in red and green, which link the two six-helix origami nanotubes. The blue tubular helix shown in the upper right of the figure represents the DNA scaffold strands which are represented by turquoise cylinders. Staple strands, except for the cross-linking strands, were omitted for clarity.
2.6.1.2 DNA Sequences

The complete list of staple strands used to construct the nanotubes is provided in Ref. 1. A list of specific staple modifications that were made to the original six-helix bundle staples is shown in Table 2.1. The modifications for each waveguide design are displayed horizontally across the table. The vertical columns represent the helix positions of each staple that required modification, with the exception of the “other modifications column,” while the numbers in the column represent the specific staple column. Only staples that were modified are shown. Blue and green numbers represent the addition of “A” or “B” stickyends to the 3’ end of the original staples, respectively. Red and purple numbers represent staples that were extended by or shortened by 14 nucleotides, respectively. The staples were shortened on the 5’ end while extensions were added to the 3’ end.
Table 2.1. A list of staple strands that were modified from the original six-helix bundle nanotube design. The waveguide design name is listed in the left most column. The color-coded numbers in the remaining columns represent the location and type of modification performed on the particular waveguide designs. Only staple strands requiring modification are shown. The specific modifications are color-coded according to the key found at the bottom of the table.

<table>
<thead>
<tr>
<th></th>
<th>Helix 1</th>
<th>Helix 4</th>
</tr>
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<td>4, 22, 40, 58, 76</td>
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<td>2, 11, 20, 29, 38, 47, 56, 65, 74, 83</td>
<td>4, 13, 22, 31, 40, 49, 58, 67, 76, 85</td>
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<td></td>
<td>2, 20, 38, 56, 74</td>
<td>4, 22, 40, 58, 76</td>
</tr>
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</tr>
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<tr>
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<td>4, 13, 22, 31, 40, 49, 58, 67, 76</td>
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</tbody>
</table>

"A" stickyend added to 3’ end of original staple strand
"B" stickyend added to 3’ end of original staple strand
Original staple shortend by 14 bases on 5’ end
Original staple extended by 14 bases on 3’ end

The “A” and “B” sticky-end sequences are 5’-ACCAGTGCTCCTACG-3’ and 5’-TCTCTACCGCCTACG-3’, respectively. The sequences were generated using a genetic algorithm designed to reduce self-complementarity and ensure a low affinity between strands.

2.6.1.3 Nanotube Synthesis

To form the DNA origami nanotubes, M13mp18 DNA scaffold strands were folded into the nanotube shape with the addition of 170 or 172 synthetic DNA staple
strands, depending on design. The staple stands were added in a 10:1 ratio of staple strands to M13mp18 scaffold strands in 1×TAE buffer solution with 12 mM MgCl2. The mixture was annealed at a constant 95 °C for 20 minutes and then cooled at a rate of 1.0 °C per five minutes. The nanotubes were purified using 0.7 - 1.0% agarose gels running at 40 volts for four hours. The gels were prepared using 0.5×TBE buffer with 12 mM MgCl2. The annealed nanotube mixture was loaded into the gel with a loading buffer consisting of 60% type 400 Ficoll solution and 40% bromophenol blue dye (both Sigma Aldrich) in a 1:4 ratio of loading buffer to the annealed DNA solution. The gels were stained for 30 minutes using a 1:10000 ratio of SYBR Gold nucleic stain (Invitrogen) to 0.5×TBE buffer with 12 mM MgCl2 solution. The gels were destained for 30 minutes using a solution of 0.5×TBE buffer with 12 mM MgCl2. The corresponding nanotube gel bands were cut out, finely chopped, and centrifuged at 4800 rcf for ten minutes at 4 °C in a Freeze ‘N Squeeze spin tube.

For synthesis of the 2xD3 nanorails, equal mole solutions of the two nanotubes were mixed together in 0.5×TBE buffer and the final MgCl2 concentration was adjusted to 40 mM. The solution was annealed at 45 °C for two hours to aid in the hybridization process. Yields of approximately 90% were observed using this method. The nanorail solution was purified using the same gel electrophoresis as described above. Then the AuNPs were attached to the nanotubes in a 5:1 ratio of AuNPs to nanotube binding sites. The sample was purified a second time to remove the unattached AuNPs using the same gel electrophoresis previously described.
2.6.1.4 Gold Nanoparticle Conjugation

The AuNPs were conjugated by adding 25 mL of 10 nm colloidal AuNP solution (BBI) to 5 mg of BSPP (Sigma-Aldrich). This step was necessary to stabilize the AuNPs at high concentrations in buffer solution containing around 10 mM MgCl$_2$. The vial containing the solution was covered in aluminum foil to protect it from light and was gently shaken for 48 hours. 5.0 M NaCl solution was added to the AuNP solution in 0.2 mL increments until a bluish purple color change was observed. The tube was centrifuged at 3200 rcf for 30 minutes and the clear supernatant was removed without disturbing the formed pellet. The remaining solution was redissolved in 400 µL of 2.5 mM BSPP and 400 µL of methanol. This solution was centrifuged at 1600 rcf for 30 minutes and the clear supernatant was removed. The AuNP pellet that formed was dissolved in the 200 µL of 2.5 mM BSPP solution. The concentration of the resulting AuNP solution was measured using a spectrometer (Agilent, Cary 5000) and given by the measured absorbance, using an extinction coefficient of 10$^8$ M$^{-1}$cm$^{-1}$ for 10 nm AuNPs. The typical concentration of AuNPs ranged from 2.0 to 3.0 mM. The disulfide bonds of the thiolated ssDNA strands were reduced by adding 45 mM TCEP (Sigma-Aldrich) to the solution, which was allowed to incubate for 30 minutes while it was gently shaken. Thiolated ssDNA was added to the AuNPs in a 300:1 ratio of thiolated ssDNAs to AuNPs. This solution was allowed to gently tumble for three days at 23 °C.

The conjugation of the AuNPs was tested by adding 1 µL of conjugated AuNPs to 10 µL of the varying concentrations (10, 30, and 100 mM) of MgCl$_2$ in 0.5×TBE. If the AuNPs fell out of solution below 30 mM, the conjugated AuNPs were not used
due to low ssDNA attachment. The unbound oligonucleotides were removed from the conjugated AuNP solution by gel electrophoresis. Purifications were performed using 0.7 percent agarose gels running at 5.6 volts per cm for one hour. The gels were prepared using 0.5×TBE buffer with 12 mM MgCl$_2$. The corresponding AuNP gel bands were cut out, finely chopped, and centrifuged at 4800 rcf for ten minutes at 4 °C in a Freeze ‘N Squeeze spin tube. After purification, the conjugated AuNPs were used immediately to ensure the AuNPs maintained the highest number of bound ssDNA possible.

### 2.6.1.5 Attachment of Gold Nanoparticles to Nanotubes

To synthesize waveguide arrays, conjugated AuNPs were hybridized to the nanotubes in a 5:1 ratio of AuNPs per binding site. To prevent site poisoning, the conjugated AuNPs were purified using gel electrophoresis to remove excess unbound DNA strands before adding the AuNPs to the unhybridized nanotubes. The mixture was annealed at 45 °C for 41 minutes to expedite the hybridization process. The hybridized nanotubes were purified using agarose gel electrophoresis to remove the excess AuNPs. Freeze ‘N Squeeze spin tubes were used to extract the waveguides from the gel. After centrifugation, the supernatant was removed and 1×TAE with 12 mM MgCl$_2$ was added to the red pellet containing the waveguides to reach the desired concentration.

Using gel purified conjugated AuNPs, we typically observed high AuNP to nanotube attachment in excess of 90 percent on well-formed waveguides of all designs. Figure 2.5 is a negatively stained TEM micrograph of the synthesized 1xD2
waveguides, which had 93.4 percent average AuNP attachment to well-formed waveguides. The average was found by visually counting the number of individual AuNPs on each waveguide and then dividing by the total number of attachment sites (in this case 18) and finally multiplying by 100 to give the percentage. Agglomerations, as can be observed in the middle of the figure, contained within the large red box, were common if the surface concentration was high. The high concentration would cause waveguides to intersect each other creating a condition in which one could not discern to which nanotube the nanoparticles were attached. For this reason we did not use entangled waveguides or waveguides that formed long chains consisting of more than one nanotube in an end to end configuration.

Figure 2.5. A negatively stained TEM micrograph highlighting the high AuNP attachment to nanotubes. The AuNP attachment of all the well-formed waveguides contained within blue is 93.4 percent. The waveguides contained within the red boxes were not used because one could not clearly discern to which nanotube the AuNPs were attached. This image represents a typical synthesis yield utilizing gel purified conjugated AuNPs.
2.6.2 TEM Staining and Imaging

Samples were prepared for imaging on thin carbon film grids (Ted Pella 01822 and 01824) that were first treated for five seconds in a glow discharge chamber built at Boise State University based on the work of Alebi and Pollard. The Tesla coil was set to the lowest setting possible while still creating discharge in approximately 3.9 Torr chamber pressure of air. Following glow discharge treatment, 2.5 µL of sample solution was deposited on the grid for five minutes, after which excess solution was wicked away using paper filter points (Ted Pella 115-18). Once dry, the sample was stained using a 2.0% w/v uranyl acetate negative stain solution that contained 25 mM NaOH. Samples were stained for 15 seconds before wicking the excess solution away using paper filter points. TEM imaging was performed at 200 keV using a JEOL JEM-2100 HR TEM equipped with a CCD camera (Orius SC1000).

2.6.3 Non-linearity of Gold Nanoparticle Waveguides

Early in the characterization of the single nanotube waveguides, AuNPs were observed to orient on both sides of the nanotubes in an uncontrolled manner as shown in Figure 2.6. This behavior was attributed to the design of the single nanotube waveguides, which consisted of only two single stranded sticky-end, attaching the individual AuNPs to the non-rigid single nanotube backbone of the waveguide. The double nanotube waveguides were designed to overcome this fundamental problem by doubling the number of sticky-ends at each binding site and adding rigidity to the waveguide by adding another nanotube.
2.6.4 AFM Images of Misaligned Laddered Waveguides

Bridging nanotubes with AuNPs can improve the rigidity of the waveguide and linearity and attachment yield of AuNPs, however, longitudinal misalignment, as shown in Figure 2.7, was observed to occur in a large number of waveguides. The
figure shows five sets of dimers being shared between two nanotubes. The independent nanotubes can be observed by examining the top portion of the waveguide shown in the figure. Guiding lines have been added to the depict the longitudinal misalignment. The formation of misaligned waveguide arrays was possible as a result of the non-specificity and translation symmetry along the long axis of the waveguide.

2.6.5 Sample Preparation for AFM and DF Characterization

Combined tographical and optical characterization of the waveguides required that they be deposited onto atomically flat and transparent substrates. A bilayered substrate was constructed consisting of a top layer of mica and a standard glass slide bottom layer. Mica was used for a top layer because it provides an atomically flat clean surface for AFM characterization and is transparent when cut into thin sheets less than a few millimeters thick. A standard glass slide (Fisher Premium 75×25 mm) was used as the bottom layer to provided structural stability to the mica and provided a convenient form factor for mounting the sample in the darkfield microscope.

The sample substrate holder was prepared by cleaning the glass slides in a staining jar filled with 2.0% Hellmanex solution and sonicated for ten minutes. The clean glass slides were removed with tweezers and rinsed with Milli-Q water to remove the Hellmanex residue. The slides were dried with ultra-high purity nitrogen gas. A 5.0 µL drop of optical epoxy (Norland, NOA61) was placed on the center of the glass slide. A 10 mm diameter mica disk (TedPella, V1) was positioned onto the drop of epoxy. The weight of the mica disk spreads the epoxy resulting in a bubble
free interface. The epoxy was cured by exposing the sample to a 365 nm wavelength light source for five minutes.

Prior to depositing the waveguide solution the mica surface was freshly cleaved. Care was taken to ensure a entire sheet of mica was removed during the cleaving process, ensuring that the surface was atomically clean prior to sample deposition. Any fragments of mica on the surface were found to interfere with the optical measurements due to their intense scattering. The waveguide solution was diluted to \( \sim 20 \text{ pM} \) with 0.5×TBE buffer with 11 mM MgCl\(_2\) to ensure the final surface concentration of waveguides was optimal for darkfield characterization. 5.0 \( \mu \text{L} \) of the diluted waveguide solution was deposited and spread over the mica surface using the pipette tip. The concentration of waveguides was critical to ensure the final surface density of waveguides was not too high as to cause excess scattering in the darkfield image and not too low, which caused difficulty in performing AFM scans. Low surface concentrations of waveguides, such as ten tubes per 30×30 \( \mu \text{m}^2 \) were found to be more desirable for registration of individual waveguides.

Following the deposition of the diluted waveguide solution, 10 \( \mu \text{L} \) of 1.0×TAE with 10 mM nickel(II) acetate was added to bind the waveguides to the mica surface. This step was found important to evenly distribute waveguides on the mica surface and to reduce the surface charge, which was found to interfere with the AFM scanning. The waveguides were allowed to adsorb onto the mica surface for five minutes followed by a 5.0 mL water rinse to remove excess salts present in the buffer. The sample was gently dried with nitrogen.
2.6.6 Gold Nanoparticle Enhancement

Increasing the scattering cross sections of the AuNPs on the waveguides was necessary to ensure a detectible scattering intensity. The waveguides were enhanced using electroless deposition (Nanoprobes, GoldEnhance Kit). In order to achieve uniform nanoparticle enhancement the decorated nanotubes were enhanced in solution. 5.0 µL GoldEnhance Solution A (enhancer) and 5.0 µL Solution B (activator) were mixed in a 0.5 mL centrifuge tube by vortexing. The solution was allowed to react for five minutes followed by the addition of 5.0 µL of GoldEnhance Solution C (initiator). The solution was again vortexed and 5.0 µL of the combined GoldEnhance solution was reacted with 5.0 µL of 1.0 nM decorated nanotubes. This mixture was vortexed and allowed to react overnight at 10 °C.

2.6.7 AFM Characterization

The waveguides described in this paper were imaged using non-contact tapping mode AFM. Non-contact tapping AFM is a variation of tapping mode AFM that utilizes low amplitude tip oscillations (approximately 1-3 nm) to minimize probe-sample interactions. This is accomplished by tuning the probe to frequencies above the peak resonant frequency (essentially limiting the probe to only experience attractive forces from the sample) and then lowering the drive amplitude to between 6-10 mV. The combination of the low amplitude tip oscillations and attractive forces allows the probe to interact with the surface without directly contacting it, which reduces tip degradation and maintains a high radius of curvature allowing for improved resolution. The large reduction in tip degradation allows for multiple large
(20 x 20 µm) images to be taken continually at high resolutions (1-4 nm) without significant loss of image resolution.

The registration process began by collecting four 20 x 20 µm AFM scans which formed the base of the combined AFM and darkfield image. A reference marker was created by placing an ink dot on the surface after sample deposition. Figure 2.8 is a schematic overview showing the relationship between the reference marker and the four AFM scans. A distinct feature of the ink dot shown in Figure 2.8 is selected using the 100 x objective on the Bruker AFM. The point selected becomes the origin, shown as the red cross-hair in the figure. The AFM mechanical stage was then moved 200 µm from the origin in the x direction, and four 20 x 20 µm scans, shown in green, with 2-5 µm overlap were collected using a programmed move. The resulting four AFM scans were then electronically stitched together using the unique patterns the waveguides created on the surface in the overlapping margins of the scans.

Figure 2.8. The relationship between the reference marker and the four AFM scans shown in green. The selected origin is directly under the red cross-hair.

2.6.8 Optical Characterization

A schematic of the optical characterization setup is shown in Figure 2.9. The microscope was outfitted with two sample stages (Nano-LPS200 and Micro-Stage,
Mad City Labs) to facilitate precise position control. The samples were excited with a Xe lamp (Shutter Instrument model LB-LS/30) through a high resolution illuminator condenser (CytoViva). A Plan Fluor 100x/0.5-1/3 oil iris objective (Nikon) was used to collect the scattering spectra with a 600 lines per mm grating and a CCD camera (Princeton Instruments, ProEM 512). A 480 nm long pass filter (Edmund Optics) was inserted after the lamp to reduce the scattering background generated by the sample substrate. The polarized scattering spectra were collected using a custom motorized polarizer that was inserted into the optical path beneath the sample prior to the spectrometer. Scattered light from the waveguides was collected through the objective lens and directed into the spectrometer. The exposure time for the CCD camera ranged from one to three minutes. To remove background signal present in the scattering spectra, spectra from an adjacent area containing no sample were subtracted from the scattering spectra. The resulting spectra were divided by the spectrum of the Xe lamp and processed.
Figure 2.9. Schematic of the experimental setup used to optically characterize the waveguides. The glass slide on which the waveguides were deposited is positioned face-down and immersion oil is placed on the top side of the slide. A Xe lamp illuminates the sample through a long-pass filter and a illuminator. Scattered light from the waveguides is collected through the objective and is then directed into a CCD spectrometer. A motorized polarizer, which is not depicted, was used to collect the polarized far-field scattering spectra from individual waveguides.

The optical characterization was performed by initially setting the grating on the spectrometer to zero to allow for the course positioning of the sample with the optical path of the microscope using the microstage controller. By referencing a fiducial mark sample, the sample was coarsely positioned into optical path of the microscope. The direction to move the inverted sample was determined by performing image transformations on a top perspective optical image collected during AFM imaging. Two image transformations, a vertical flip and a 90 degree rotation were required so the image of the sample observed on the microscope would
match the orientation of the original AFM image that marked the location of the waveguide of interest. After the course sample alignment the condenser was initially adjusted to reduce the background intensity using the 40x objective.

Figure 2.10. A schematic highlighting the process of combining the darkfield images using the unique pattern of the waveguides in the AFM scan and the pattern observed in the darkfield image. (a) The AFM scans and darkfield image before being combined. (b) The resulting combined AFM and darkfield image.
Next, the 100x objective was used to closely align to the center of the reference mark to establish the same origin as the original optical image acquired from the atomic force microscope. The condenser was again optimized to achieve the highest contrast and lowest background intensity possible. The sample stage was moved from the reference mark to the center of the previously collected AFM scan. At this point, a DF image of the entire sample area was collected using the 100x objective and the image was re-sized to match the scale of the original AFM scan. The collected DF image was then made transparent and digitally overlaid on top of the AFM image. Figure 2.10a, shows the collected darkfield image and AFM scans after being scaled and prior to being combined. The combined uncropped image is shown in Figure 2.10b. The pattern made by the waveguides on the AFM was used to visually align the two images. Once the images were aligned to each other, the selected waveguides were numbered and the individual spectra were collected.

Spectra for the individual waveguides were collected by reducing the spectrometer’s slit width to around 30 µm, setting the camera’s region of interest so that only the scattered light from the selected waveguide was collected during the scan of 480 to 700 nm. Different camera exposure times were used depending on the intensity of the waveguide being characterized. Unenhanced waveguides required long exposure times up to five minutes while enhanced waveguides usually required a three minute exposure to ensure a defined spectrum was produced. The combined AFM and darkfield images were overlaid by the following process: 1) The original 20×20 µm² AFM scan was processed in Bruker’s NanoScope Analysis software by performing a plane fit and then flattening the scan; 2) An inverted grayscale image
was overlaid on top of the AFM scan with a transparency setting ranging from 40-60% depending the contrast of the darkfield image.

2.6.9 Scattering Spectra from Single Nanotube Waveguides

Approximately 20 1xD1 waveguides were independently characterized throughout this study. The scattering spectra collected from several of the 1xD1 waveguides are shown in Figure 2.11. The combined spectra of the selected waveguides is shown in Figure 2.11e. The combined AFM and darkfield image is shown in Figure 2.11f with a schematic of the waveguide design shown in the inset of the figure. The scattering spectra collected from the 1xD1 waveguides were generally not well defined due to the non-linearity of the waveguide. Despite the generally poorly defined spectra, the bulk plasmon resonance of the independently collected waveguides did correlate with each other as is shown in Figure 2.11e.
Figure 2.11. (a-d) The scattering spectra collected from several 1xD1 waveguides on the surface of mica. The individual spectra were collected from the respective waveguide locations shown in (f). A schematic diagram of the 1xD1 waveguide is shown in the inset of (f). (e) The combined far-field spectra from NTs 1-4. The peak plasmon resonance from the waveguides varies within a relatively small range despite the large variations in the profiles of the independently collected scattering spectra. This trend was observed in all waveguide designs.

2.6.10 Single Nanotube Dimer Waveguides Scattering Spectra

The 1xD2 waveguide doubled the number of AuNP dimers from the 1xD1 waveguide to ten sets. The additional sets of dimers increased the scattering signal
making the collection of well defined spectra possible without AuNP enhancement. However, well defined spectra were easier to obtain when the AuNPs were enhanced. The individual far-field scattering spectra, combined far-field spectra, and combined AFM and darkfield image from 1xD2 waveguides are shown in Figure 2.12. The scattering spectra from the 1xD2 waveguides were generally well defined due to the stronger scattering signal as a result of doubling the number of dimers in the waveguide design and due to the AuNP enhancement of the waveguides, which increases the scattering cross section of the AuNPs. While some variations in the spectral profiles still existed the profiles of the independently collected spectra are similar to each other as can been observed in Figure 2.12e. The deviations in the peak positions between 1xD2 waveguides were attributed to the non-perfect periodicity of the independent dimers on the waveguide. The polarized spectra from these structures were noisy due to the small cross-section of the individual AuNPs. The small scattering cross section resulted in a low signal to noise ratio. Two tether strands per binding site did not always provide the structural rigidity required to hold the individual AuNPs in the designed dimer shape. The lack of rigidity led to variations in spacing between individual AuNPs, which led to variations in the waveguide’s transverse and longitudinal modes.
Figure 2.12. (a-g) The far-field scattering spectra collected from several 1xD2 waveguides. The individual spectra were collected from the respective waveguide locations shown in the combined AFM and optical darkfield image (f). A schematic diagram of the 1xD2 waveguide is shown in the inset of (f). The profile of the independently collected scattering spectra are observed to improve by doubling the number of dimers on the waveguides. (e) The combined spectra from all the waveguides shown in (f).
2.6.11 Single Nanotube Timer Waveguides Scattering Spectra

The 1xT waveguide was designed to have five sets of AuNP trimers along the longitudinal axis of the nanotube. The trimers were expected to cause a red-shift in the scattering spectra due to the elongated longitudinal dimensions of the AuNP trimer set causing a shift in the plasmon resonance. The individual far-field scattering spectra, combined far-field spectra, and combined AFM and darkfield image from 1xT waveguides are shown in Figure 2.13. The expected red-shift resulting from the longitudinal mode of the trimers was observed and was found to be consistent with the calculated values. The spectral profile of the 1xT waveguides generally agreed with one another but deviation occurred due to the non-linearity of the single nanotube design.
Figure 2.13. (a-e) The scattering spectra collected from several 1xT waveguides. (f) The combined AFM and optical darkfield image depicting the locations of the waveguides. A schematic diagram of the 1xT waveguide is shown in the inset of (f). The expected red-shift in the plasmon resonance was observed. (g) The combined spectra from all the waveguides shown in (f).

2.6.12 Single Nanotube Waveguides Polarized Scattering Spectra

Polarized far-field scattering spectra were collected from the 1xD1 and 1xD2 waveguides; however, the polarization dependency of the waveguides’ scattering
was poorly defined due to the non-linearity and misalignment of the individual dimer sets. Polarization dependent scattering spectra collected from the 1xD1 and 1xD2 waveguides are shown in Figure 2.14a and 2.14b, respectively. AFM images of the characterized waveguides are shown to the left of the respective spectra. Black lines have been added to the AFM images to show the deviations in orientation between dimer sets on the individual waveguide structures.

Figure 2.14. The polarized scattering spectra collected from (a) 1xD1 and (b) 1xD2 waveguides shown in the height AFM images to the left of the spectra. The TM mode, shown in blue, and LM mode, shown in red, were collected with the polarizer perpendicular to and parallel to the long-axes of the waveguides, respectively. The spectra did not correlate to the calculated spectra due to the inconsistent alignment of the independent dimers along the long axis of the waveguide. Black lines have been added as a visual aid to show the degree of misalignment.

2.6.13 Laddered Nanotube Waveguides Scattering Spectra

The 2xD2 waveguide consisted of ten AuNP dimer sets held in place by two nanotubes. The added constraint of the second nanotube substantially, increased the
linearity of the waveguides which led to clean, well-defined scattering spectra as shown in Figure 2.15. By closely studying the individual scattering spectra one can see that the large peak is comprised of two independent overlapping peaks. The center of the first peak around 575 nm and the second around 600 nm correspond to the transverse and longitudinal plasmon resonance modes of the waveguide, respectively.

Figure 2.15. (a-c) The far-field scattering spectra collected from three 2xD2 waveguides. The individual spectra were collected from the respective waveguide locations shown in (d). (d) The combined AFM and optical darkfield image. A schematic diagram of the 2xD2 waveguide is shown in the inset of (d). (e) The combined far-field spectra from NTs 1-3. The tops of the traverse and longitudinal mode peaks are evident in the scattering spectra.

2.6.14 Cross-Linked Nanotube Waveguides Scattering Spectra

The 2xD3 waveguide consisted of ten AuNP dimer sets similar to the 2xD2, however the nanotubes were cross-linked to each other, which added increased
linearity and eliminated the formation of misaligned waveguides. The individual far-field scattering spectra, combined far-field spectra, and combined AFM and darkfield image from 2xD3 waveguides are shown in Figure 2.16. The independent scattering spectra are well defined and the peak position agrees with the expected value for AuNP dimer sets. Independently collected spectra from three 2xD3 waveguides all show well defined spectra and thus indicate that two nanotubes can provide the necessary structural stability required to construct reliable waveguiding structures.

Figure 2.16. (a-c) The far-field scattering spectra collected from three 2xD3 waveguides. The individual spectra were collected from the respective waveguide locations shown in (d). (d) The combined AFM and optical darkfield image. A schematic diagram of the 2xD3 waveguide is shown in the inset of (d). (e) The combined far-field spectra from NTs 1-3.
2.6.15 Waveguide Linearity and Inter-particle Spacing Measurements

The linearity of the synthesized waveguides was analyzed by measuring the length of the line constructed tangent to the end of a waveguide and extending to the point where the curvature of the waveguide visually deviated from the center of the drawn line, as is shown in Figure 2.17. Lines were constructed beginning from each end of the waveguide. The two lengths were then averaged to give the average linearity for each waveguide.

![Figure 2.17](image)

Figure 2.17. A height AFM image depicting how the average linearities of the waveguides were determined. Two lines (green and pink) were drawn tangent to the end of the waveguide. The point where the nanotube deviated from the center of the drawn line was set as the end of the line. The total length of the line was then measured from the tangent beginning point to the set end point. The two line lengths, in the case above 361 and 204 nm were averaged to give an average tube linearity, 282 nm.

The inter-particle spacing of the waveguides was quantitatively determined by averaging the distance between successive nanoparticles contained within the dimer and trimer sets. The average length between successive dimer and trimer sets is shown in Table 2.2.
Table 2.2. A table comparing the designed spacing between successive nanoparticle dimer and trimer sets to the spacing observed in the synthesized waveguides.

<table>
<thead>
<tr>
<th>Waveguide Design</th>
<th>Designed Spacing Between Sets (nm)</th>
<th>Average Space Between Sets (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1xD1</td>
<td>70</td>
<td>61 ± 10</td>
</tr>
<tr>
<td>1xD2</td>
<td>29</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>1xT</td>
<td>56</td>
<td>56 ± 6</td>
</tr>
<tr>
<td>2xD1</td>
<td>28</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>2xD2</td>
<td>28</td>
<td>30 ± 5</td>
</tr>
</tbody>
</table>
REFERENCES


CHAPTER THREE: DNA ORIGAMI FRET-BASED PHOTONIC WIRE

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3.1 Abstract

Self-assembled DNA photonic wires are a potential candidate to be used in nanoscale communications, biosensing, and in light harvesting applications. Molecular photonic wires exploit Förster Resonance Energy Transfer (FRET) to transfer energy through nonradiative dipole-dipole interactions. Recently, homogeneous FRET (homoFRET) has emerged as a potential way of increasing photonic wire energy transfer efficiency. However, little is known about the basic design principles needed to construct synthetic multistep photonic devices that incorporate homoFRET despite its well-known occurrence in natural photosynthesis. In this work, we attempt to address this knowledge gap by designing, constructing, and characterizing a DNA photonic wire with a 10-dye reconfigurable homoFRET center. The DNA labeled fluorophores were attached inside a six-helix bundle nanotube to form a 30 nm long photonic wire. Over 50 wire configurations were characterized using steady-state and time-resolved fluorescence measurements. Several wire configurations were shown to have increased the end-to-end efficiencies. A ∼ 200% increase in the end-to-end to efficiency was observed when the first fluorophore was removed from the full wire, as compare to the full functionalized wire.

3.2 Introduction

Multistep FRET photonic wires have applications ranging from energy transport, biosensing, and photonic networks to facilitate nanoscale communications. Photonic wires are similar to optical waveguides in the sense that light energy is
transferred linearly from one end to the other. One application of multistep FRET is in the construction of photonic wires which could function as nanoscale information transfer devices. In FRET-based photonic wires, fluorophores are used in place of materials such as gold nanoparticles. A scaffolding molecule, such as DNA, is used to arrange the fluorophores. DNA origami self-assembly has been shown to be a viable method for the bottom-up assembly of nanoscale devices. The technique has been used in the past to arrange functionalized or labeled nanomaterials such as gold nanoparticles, quantum dots, and fluorophores with nanoscale precision.

Energy harvesting devices exploit FRET to transfer energy through dipole-dipole interactions between donor and acceptor molecules. The energy transfer is termed heterogeneous transfer when energy is transferred between different fluorophores. In contrast, the term homoFRET is used to describe the energy transfer between the same fluorophores. Both transfers are dependent on the sixth power of the distance between the donor and acceptor. In theory, homotransfer is energetically lossless. Homotransfer was first observed in connection with the fluorescence depolarization in solutions with increasing dye concentration. It is used in natural photosynthesis to transfer energy to reaction centers with high efficiency, (>90%).

Previous work has shown homoFRET can be used to bridge between a donor and acceptor molecule and effectively increase the distance over which energy can be transferred. Three and five dye molecular photonic wires, which incorporated homoFRET regions of up to six homoFRET dyes, have been demonstrated. In this work, homoFRET simulations revealed that shorter distances between the donor and
acceptor fluorophores resulted in a more efficient transfer. If the distance between successive homoFRET dyes could be reduced to less than half the Förster radius, then a 30 nm long photonic wire should be capable of an end-to-end transfer efficiency of >5%. However, it is important to recognize that the FRET efficiency has a distance dependence to the sixth power, but the number of steps to transverse the homoFRET section of the MPW increases to the second power.\textsuperscript{12}

In this investigation, we set out to study the effect that the homoFRET dye configuration has on the overall wire energy transfer efficiency. In the past, most DNA assembled photonic wires were constructed with relatively short (<100 nucleotides) synthetic DNA oligos as the DNA scaffold.\textsuperscript{12,16} The technique works well for relatively small structures consisting of less than about 300 base pairs. However, if the method is used to create larger DNA structures the formation yield begins to decrease as a result of the competition between multiple synthetic template strands.\textsuperscript{2} DNA origami overcomes this problem by using a single scaffold. In this work a 704 nucleotide plasmid was prepared by cleaving the commercially available M13mp18 plasmid. The shorter scaffold allowed for the formation of origami with about 1/10th the size of an origami folded with a typical M13mp18 scaffold. Using the shorter scaffold we constructed a 30 nm long linear photonic wire consisting of three unique fluorophores, Alexa Fluor 488 (AF488), Cy3.5, and Alexa Fluor 647 (AF647). The wire consisted of two AF488 input dyes, a reconfigurable ten Cy3.5 homoFRET region, and two terminal AF647 dyes. The fluorophores were arranged inside a six-helix bundle origami.
3.2.1 Photonic Wire Design

The design is shown in Figure 3.1. Figure 3.1a is a two-dimensional depiction of the three-dimensional vHelix model used to aid in design of fluorophore attachment locations. The DNA origami specificity allowed for the individually addressable Cy3.5 dye locations. Creating devices with individually addressable Cy3.5 dye locations was not possible with previous photonic wires, which used repeating sequence domains to form the homoFRET regions. The disadvantage of this approach is increased cost and variability in dye labeling associated with each DNA sequence. Figure 3.1b shows the scaffold and staple routing of the wire and indicates the fluorophore attachment locations adapted from CaDNAno software. The blue, green, and red dots represent the AF488, Cy3.5, and AF647 attachment locations, respectively.

The fluorophores were attached to the 5' end of each staple strand. The attachment locations for the fluorophores were carefully selected to ensure their attachment locations would be oriented in the center of the six-helix bundle nanotube. The AF488 and AF647 fluorophores were attached to helix 1 and 2. The Cy3.5 fluorophores were attached to helices 1, 2, and 3, as can be seen in Figure 3.1b. The intent was that the fluorophore linker chemistry would position the dyes in approximately the axis of the six-helix bundle, thus forming a straight photonic wire. To represent the specific wire configurations’ the naming convention is shown in Figure 3.1c was adopted. A number represents that the dye is present at the respective location whereas an underscore dash, "_" indicates that the unlabeled strand has been used. Dashes, "-" signify the boundary between different
fluorophores domains. For example, the full wire is represented as 12-0123456789-12 and wire missing alternating Cy3.5 dyes is represented as 12-1357912, as shown in Figure 3.1c. The number one was not used to represent the presence of the first Cy3.5 fluorophore since there was a total of ten Cy3.5 dyes and the tenth dye would have to be represented by a two-digit number or a non-numeric symbol.

Figure 3.1. Schematics depicting the location of the fluorophores in the photonic wire and notation used to describe them. (a) The helix model of the six-helix bundle photonic wire with the fluorophore locations shown as blue, green, and red dots for the AF488, Cy3.5, and AF647 fluorophores, respectively. (b) The wire’s scaffold and staple routing adopted from CanDNAo. The fluorophore attachment locations are on the inside of the six-helix bundle. (c) The simplified wire notation was developed to describe the particular wire configurations. Two wires are given as an example of the notation. The top wire contains all the fluorophores whereas the lower wire’s homoFRET region consists of alternating Cy3.5 dyes.
3.3 Methods

3.3.1 Materials

Labeled and unlabeled DNA strands were obtained from Integrated DNA Technologies (Coralville, USA) and Eurofins Operon (Huntsville, USA). The sequences of the DNA can be found in Tables 3.3 and 3.4. All dye labeling was performed on terminal bases, succinimidyl ester labeling with six carbon linker was used for AF488 and AF647 whereas double phosphoramidite labeling was used for the Cy3.5. The quantum yield (QY) of each dye was experimentally determined using fluorescent standards. Fluorescein, Rhodamine 640 (Rhodamine 101), and Rhodamine 800 (LD 800) were used as the standards for, AF488, Cy3.5, and AF647, respectively.19

3.3.2 Fluorophore Photophysical Properties

The overlap integrals \( J \) and Förster distances \( R_0 \) for the independent dyes were calculated using Equations (3.1) and (3.2), respectively. Where, \( I_D \) is the donor emission spectrum normalized to unity, \( \epsilon_A(\lambda) \) is the extinction coefficient and \( \lambda \) is the wavelength in units of nm.20 Where, \( n \) is the refractive index, set to 1.33 for 2.5x PBS buffer, \( \Phi_D \) is the fluorescence quantum yield of the donor and \( \kappa \) is the dipole orientation taken to be 2/3, and \( J \) is the overlap integral in units of \( (nm^4 * M^{-1} * cm^{-1}) \).20

\[
J = \int I_D(\lambda) \epsilon_A(\lambda) \lambda^4 d\lambda \quad (3.1)
\]

\[
R_0 = 0.02108 [\kappa^2 \Phi_D n^{-4} J]^{1/6} \quad (3.2)
\]
The J integrand depends on both the absorption spectrum of the acceptor and the emission spectrum of the donor. It is a measure of the degree of spectral overlap between donor-acceptor pairs. The absorption and emission spectra for the fluorophores are shown in Figure 3.2.

![Fluorophores Absorbance and Emission Spectra](image)

**Figure 3.2.** The normalized absorption and emission spectra for the three fluorophores used in the photonic wire. The spectra for the AF488, Cy3.5, and AF647 dyes are shown as blue, green, and red lines, respectively. The absorption and emission spectra are depicted as dashed lines and solid lines, respectively.

### 3.3.3 Synthesis

The DNA origami was prepared in 1×TAE buffer with 15 mM Mg$_{2+}$. The final DNA origami concentration was set at 10 nM. The samples were annealed in a PCR cycler. The DNA anneal program used to fold the DNA origami photonic wire was developed by analyzing the origami’s melting curve. A qPCR machine was used in an adapted technique to determine the origami’s hybridization temperatures. The qPCR melting curve showed three peaks at 76 °C, 70 °C, and 49 °C which correlated with an increased rate of DNA hybridization. The developed anneal program can be found in Section 3.8.7. The program was designed to hold these three temperatures.
for relatively long periods. Additionally, to reduce the likelihood of dye degradation the time spent at elevated temperatures above 77 °C was minimized. To reduce the effects of photobleaching and dye degradation, all samples were stored in the dark at 4 °C. Agarose gel electrophoresis was used to evaluate the constructs formation yield.

3.3.4 Steady-State Absorption and Fluorescence Measurements

The devices steady-state absorbance and fluorescence measurements were collected to assess the transfer efficiency and antenna gain. The absorption and fluorescence spectra of the individual fluorophores were measured and used to calculate the overlap integrals and Förster radius for each fluorophore. The absorption spectra were collected using Agilent 8453 diode array UV-Vis spectrometer. In comparison, the steady-state fluorescence spectra was collected using a Tecan Infinite M1000 dual monochromator (Tecan, Research Triangle Park, USA). The samples were excited at 466 and 535 nm in microtiter 96-well plates. The excitation wavelengths were chosen to directly excite the AF488 and Cy3.5 fluorophore, respectively. 100 flashes at a gain of 200 was sufficient to collect spectra with an adequate signal-to-noise ratio. The sample volume was set at 100 µL with a device concentration set at 0.20 µM. The fluorescence spectra was collected with a 1 nm step size through a 500-880 nm range. The flash frequency was 400 Hz and the integration time 40 µs. All of the collected fluorescence spectra were smoothed using a five-point running average before performing a least-squares fit analysis to fit the collected emission spectra. The fit was then deconvoluted to extract the individual fluorophore components. The component areas were then used to calculate the distances between the fluorophores and the wire’s transfer efficiency.
3.3.5 Fluorescence Lifetime and Fluorescence Lifetime Anisotropy

Fluorescence lifetime and fluorescence lifetime anisotropy measurements were collected to better understand the energy transfer and to confirm the presence of homoFRET. A time-correlated single photon counting (TCSPC) technique using a Becker-Hickl SPC-630 board was used. The excitation laser was a 80 MHz, 7 ps pulsed, 532 nm source. The experimental setup is described further in the following citations.\textsuperscript{22,23} The standard fluorescence lifetime measurements were conducted by exciting the samples with a pulse of polarized light. The samples fluorescence was observed through a polarizer set to the magic angle and then filtered through a monochromator. The change in fluorescence intensity of the sample was then recorded. The device’s intensity was assumed to decay as the sum of individual single exponential decays. The lifetimes were found by fitting a multi-exponential decay function to the collected fluorescence decay spectra. The average of the two lifetimes was taken to be the device’s overall decay rate. The multi-exponential decay functions are given in Section 3.8.9.

Anisotropy measurements are typically used to reveal the fluorophores average angular displacement or rotational correlation time that result due to the molecules rotational diffusion. However, the method can also be used to detect the presence of homoFRET which is indicated by a decrease in the samples polarization or anisotropy. The additional decay pathways arising from homoFRET cause the sample’s anisotropy to decrease. The anisotropy measurements were conducted by exciting the sample with a pulse of polarized light. The fluorophores whose dipoles are aligned parallel to the polarization are excited and then at a later time re-emit the
light at a different angle. The anisotropy is measured by adjusting the polarizer to parallel and perpendicular alignments.

3.3.6 Transfer Efficiency

The efficiency is a measure of the number of photons emitted by the terminal Cy5.5 fluorophores to the number of photons introduced at the AF488 fluorophores. The efficiency also accounts for the quantum yield of the donor and final AF647 acceptor fluorophores. Past work has used what we call the anywhere-to-end efficiency, \((E_{ae})\) which does not correct for the direct excitation that occurred throughout the wires in the Cy3.5 homoFRET region. The \(E_{ae}\) is defined as Equation (3.3). To evaluate the energy transfer of the wires we used three metrics which represents the transfer efficiency. An anywhere-to-end \((E_{ae})\), an end-to-end efficiency \((E_{ee})\), and the wire transfer efficiency (WTE) were calculated for each wire.

The \(E_{ae}\) is defined as Equation (3.3). Where \(\phi_{AF647\ and\ AF488}\) is the integrated area of the peak component for the terminal acceptor in the presence of donor, \(\phi_{AF647}\) is the integrated fluorescence area of the terminal acceptor alone, \(\phi_{AF488}\) is the integrated fluorescence area of the donor in the absence of any acceptor, and \(\Phi_{AF647}\) and \(\Phi_{AF488}\) are the quantum yields (QY) of the terminal acceptor and donor, respectively.\(^{24}\)

\[
E_{ae} = 100 \times \frac{[(\phi_{AF647\ and\ AF488} - \phi_{AF647})/\Phi_{AF647}]}{\phi_{AF488}/\Phi_{AF488}}
\]  

\[\text{(3.3)}\]

In addition to calculating the \(E_{ae}\) we calculated a value for the experiment
corrected efficiency ($E_{ee}$). The $E_{ee}$ was calculated to give a true measure of the direct excitation that occurred throughout the constructs but especially in the Cy3 homoFRET region. The $E_{ee}$ was defined as Equation (3.4). Where $\phi_{AF647_{466}}$ and $\phi_{AF647_{585}}$ are the integrated fluorescence area of the terminal AF647 acceptor in the presence of all dyes excited at 466 nm and 585 nm, respectively. $\phi_{AF488}$ is the integrated component area of the donor in the absence of any acceptor, and $\Phi_{488}$ and $\Phi_{AF647}$ are the QYs of AF488 and AF647, respectively. $\Delta_{CF}$ is the correction factor calculated experimentally.

$$E_{ee} = \frac{100 \left[ \phi_{AF647_{466}} - (\Delta_{CF} \phi_{AF647_{585}}) \right]}{\phi_{AF488}/\Phi_{AF488}}$$

(3.4)

$$\Delta_{CF} = \frac{\phi_{AF647_{466}}^{0123456789\text{-}12} \phi_{AF647_{585}}^{0123456789\text{-}12}}{\phi_{AF647_{466}}^{0123456789\text{-}12} \phi_{AF647_{585}}^{0123456789\text{-}12}}$$

(3.5)

3.3.7 Wire Transfer Efficiency

One of the goals of this investigation was to determine what effect that increasing dye density had on the number of photons transferred along the wire independent of variations in input. We developed another way of looking at the transfer efficiency that is independent of the number of photons put into the wire. We defined the wire transfer efficiency (WTE) given by Equation (3.6). Where $\phi_{Cy3.5}$ is the emission based on the specific AF488-Cy3.5 design with no additional Cy3.5 once FRET efficiency was saturated with no final acceptor. We observed that the FRET saturated after three steps so in the case of the full wire, 12-0123456789-12, the $\phi_{Cy3.5}$ sensitized emission from __-012_______-12 formed our baseline.

$$WTE = 100 \times \left[ \frac{(\phi_{AF647 \text{ and } AF488} - \phi_{AF647}) / \Phi_{AF647}}{\phi_{Cy3.5} / \Phi_{Cy3.5}} \right]$$

(3.6)
3.3.8 FRET Step Efficiencies

The step efficiencies for the individual energy transfer steps in the wire were calculated by collecting the fluorescence spectra from wires comprised of only the individual dye pairs. Unlabeled DNA strands replaced the original labeled strands when the dyes were not present. The efficiencies were calculated using Equation (3.7) and is shown in Figure 3.7. These values were used in the Monte Carlo simulations and to better understand the wire’s energy transfer. Where $F_{DA}$ is the fluorescence intensity of the donor in the presence of acceptor, $F_D$ is the fluorescence intensity of the donor in the absence of acceptor.

$$E_D = 1 - \frac{F_{DA}}{F_D}$$  \hspace{1cm} (3.7)

The step efficiencies for the individual energy transfer was also calculated using Equation (3.8) which accounts for donor quenching and acceptor sensitization. Where $\Phi_A$ and $\Phi_D$ are the QYs of acceptor and donor, respectively, $F_{DA}$ is the fluorescence intensity of the donor in the presence of acceptor, $F_D$ is the fluorescence intensity of the donor in the absence of acceptor and $F_{AD}$ is the luminescence intensity of the acceptor during FRET.$^{20}$

$$E_{DA} = \left[1 + \frac{\Phi_A}{\Phi_D} \frac{F_{DA}}{F_{AD} - F_A}\right]^{-1}$$  \hspace{1cm} (3.8)

3.3.9 Monte Carlo Simulations

Monte Carlo simulations were conducted to better understand the energy transfer process. The simulations assumed that the fluorophores interacted solely via
point dipole-dipole coupling. The DNA was modeled as three parallel straight arms with dyes directed perpendicular to the DNA and with azimuthal angle directed into the center as is shown in Figure 3.12a. The dye linker length was assumed to be 0.75 nm. The dyes were allowed to move randomly swing freely at their attachment points. Static dipoles were assumed. Dyes that were not incorporated were assumed to remain in solution. The model has been previous described so only a brief description will be presented here.\textsuperscript{24,25} Since we treated target structures as incompletely formed and accompanied by assorted partial constructs/free dyes, we normalize the governing rate equations by total concentration and the variables then become equivalent to probabilities. For steady-state, only the time-integrated probability $W_{ik}$ that the $i$th dye on the $k$th construct will be excited is needed, and one can show that this obeys:

$$W_{ik}\left[1 + \sum_{j=i}^{N_k} b_{ij}^k\right] - \sum_{j=1}^{N_k} b_{ij}^k W_{jk} = \sum_{m=1}^{M} \Delta_{ik}^m \eta_m, i = 1, ..., N_k, k = 1, ..., S \tag{3.9}$$

where $S$ is the number of different constructs in the ensemble, $M$ is the number of different types of dyes, $N_k$ is the number of dyes in the $k$th construct, $\Delta_{ik}^m$ is unity only if dye $i$ on construct $k$ is of type $m$. The matrix element $b_{ij}^k$ specifies the excitonic coupling between dyes $i$ and $j$ on construct $k$, and according to Förster theory it varies as $1/r^6$ ($r=inter$-dye distance). The quantities in Equation (3.9) are related to the fluorescence intensity of each dye $\Phi_m$ by:

$$\Phi_m = \Psi_Q m \left[1 + \sum_{k=1}^{S} \frac{\rho^{(k)}}{\rho^{ideal}} \sum_{i=1}^{N_k} \Delta_{ij}^m W_{jk} + \left(N_{m,\text{ideal}}^{(1)} - \sum_{k=1}^{S} \frac{\rho^{(k)} N_k^m}{\rho^{ideal}} \right) \eta_m \right] m = 1, ..., M \tag{3.10}$$
where $Q_m$ is the quantum yield of dye $m$, $\rho(k)$ is the molar concentration of construct $k$, $N_{km}^m$ is the number of dyes of type $m$ in construct $k$.

### 3.4 Results

The formation yield of the devices was evaluated by gel electrophoresis and Atomic Force Microscopy (AFM). AFM scans of the formed origami wire, post Amicon purification are shown in Figure 3.3. The measured wire dimensions were shown to be in agreement with the wires design dimensions. Relatively high origami formation efficiency was observed.

![AFM scans of the formed wire. (a) A wide area AFM scan of the formed wires after Amicon purification. The samples were deposited on freshly cleaved mica. (b) A close-up AFM scan of the formed wires.](image)

High origami formation efficiency was also confirmed by the agarose gel electrophoresis. A 1% agarose gel of the origami wire formed at three different magnesium concentrations 10, 15, and 20 mM magnesium and is shown in Figure 3.4. It was concluded that 15 mM magnesium gave the best-formed origami as is evident
by a slightly faster migration speed through the gel. A slight secondary structure was observed as is evident by the faint bands appearing above the formed origami wire bands. Some device clumping was observed. However, device clumping was only observed when the devices were stored at 4 °C for a few days as was the case with the samples awaiting AFM characterization. Allowing the samples to equilibrate at room temperature provided a simple solution.

![Origami Formation Gel](image)

**Figure 3.4.** A 1% agarose gel, before Amicon purification, used to determine the origami’s optimal folding magnesium concentration. The M13mp18 cut is in the left most gel band whereas the full M13mp18 is shown as the rightmost band. The origami wire folded with 15 mM of magnesium and migrated slightly faster than origami wires folded with 10 and 20 mM of magnesium.

### 3.4.1 Measured Photophysical Properties

The photophysical properties of the fluorophores were calculated using the collected absorbance and fluorescence spectra for each fluorophore as described in Section 3.3.2. Table 3.1 lists the experimentally determined quantum yields (QY), the
extinction coefficients ($\epsilon$), the wavelengths for the fluorophores peak absorbance ($Abs_{\text{max}}$) and emission ($Em_{\text{max}}$), the overlap integral ($J$), and the Förster radius ($R_0$) for each fluorophore. The plots used to determine the quantum yields are contained in the Section 3.8.1.

### Table 3.1. Photophysical and FRET properties of the fluorophores.

<table>
<thead>
<tr>
<th></th>
<th>Overlap Integral $J(\text{nm}^4\text{M}^{-1}\text{cm}^{-1})$ and $R_0$ (nm) (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QY (^a)</td>
</tr>
<tr>
<td>AF488</td>
<td>0.97</td>
</tr>
<tr>
<td>Cy3.5</td>
<td>0.58</td>
</tr>
<tr>
<td>AF647</td>
<td>0.53</td>
</tr>
</tbody>
</table>

\(^a\) The average experimentally determined QY for each DNA labeled fluorophore.

\(^b\) Förster distances ($R_0$) were calculated assuming a 2/3 value for $\kappa$ and 1.33 value for $n$.

#### 3.4.2 Individual Cy3.5 Fluorophore Addressability Experiment

As an initial test of the addressability of the Cy3.5 homoFRET region, control wires were prepared with individual Cy3.5 dyes at each possible binding location. The Cy3 dye addressability was evaluated to test whether single Cy3 dyes could be attached at each Cy3 binding location within the homoFRET region of the photonic wire. Steady-state fluorescence spectra collected after Amicon purification is shown in Figure 3.5a. We observed a consistent fluorescence signal from wires composed of single Cy3.5 fluorophores demonstrating control over individual dye accessibility within the homoFRET region. Additionally, we formed wires with successively increasing numbers of Cy3.5 repeats, as can be seen in Figure 3.5b. We observed a generally linear increase in fluorescence. Some fluctuations in the fluorescence intensity were observed as the homoFRET region was lengthened. The fluorescence
of the wire with seven Cy3.5 fluorophores was lower than expected relative to the wires composed of six and eight Cy3.5 fluorophores.

![Graph](image)

Figure 3.5.  (a) A plot of the Cy3.5 fluorescence intensity collected from wires with individual Cy3.5 fluorophores attached at each successive binding location in the wires homoFRET region. (b) A plot of the Cy3.5 fluorescence intensity collected as the number of Cy3.5 fluorophores is successively increased. Results show the Cy3.5 binding locations are individually addressable.

3.4.3 Fluorescence Lifetime and Anisotropy Measurements

As was discussed in Section 3.3.5, the presence of homoFRET can be detected as a decrease in anisotropy. The average lifetime of the Cy3.5 fluorophores was found to remain relatively constant (±0.31 ns) as the homoFRET section was extended from
one to three Cy3.5 fluorophores. Anisotropy measurements on longer homoFRET configurations were not conducted. The lifetime decay fits can be found in Section 3.8.9. In contrast, the anisotropy decayed from 1.5 to 0.97 nm as the homoFRET region was lengthened to three Cy3.5 repeats. The relatively unchanged lifetime along with the decreasing anisotropy demonstrated that the wire’s homoFRET section was transferring energy through homogeneous energy transfer. The calculated fluorescence lifetime anisotropies are shown in Table 3.2. The experimentally collected fluorescence lifetime and anisotropy decay curves and fits are shown in Figure 3.6.

![Fluorescence Anisotropy Lifetime](image)

**Figure 3.6.** The fluorescence lifetime anisotropy spectra collected from three wire configurations with increasing Cy3.5 from one to three dyes. The dashed lines represent the measured anisotropies. The solid lines are the double exponential decay fits. The decreasing decay rates confirm the presence of HomoFRET.
Table 3.2. The experimentally determined anisotropy decay rates for the photonic wires. The decreasing decay rates observed confirm the presence of HomoFRET.

<table>
<thead>
<tr>
<th></th>
<th>τ₁ (ns)</th>
<th>τ₂ (ns)</th>
<th>Adj. R-Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.123 ± 0.006</td>
<td>1.5 ± 0.3</td>
<td>0.9459</td>
</tr>
<tr>
<td>45</td>
<td>0.076 ± 0.008</td>
<td>1.3 ± 0.1</td>
<td>0.9269</td>
</tr>
<tr>
<td>456</td>
<td>0.044 ± 0.006</td>
<td>0.97 ± 0.05</td>
<td>0.9230</td>
</tr>
</tbody>
</table>

3.4.4 FRET Step Efficiencies

The step efficiencies for the individual energy transfer steps are shown in Figure 3.7. The step efficiencies were calculated using the observed donor quenching and are depicted as green bars. Care should be taken when comparing efficiencies of wires composed of single donors vs. multiple donors to those with multiple donors since less donor quenching can occur when multiple donors are present. However, the trends observed are important for explaining the wires transfer efficiency. For example, we noted that the 12-0-12 wire only had a slightly higher step efficiency than 12-1-12. Their respective transfer efficiencies were 49.3 and 40.7% as can also be seen in Figure 3.7.
3.4.5 Energy Transfer Efficiency

The WTE, $E_{ae}$, and $E_{ee}$ were calculated for all the full wires configurations investigated. The first set consisted of wires in which a single Cy3.5 dye was removed from the homoFRET region. It was found that the energy transfer of the wire missing the first Cy3.5 fluorophore, 12−_123456789−12 exhibited the highest energy transfer efficiency. A plot of the calculated efficiency is shown in Figure 3.8. A
plot of the calculated WTE, $E_{ae}$, and $E_{ee}$ for the wires which exhibited the highest transfer efficiency are shown in Figure 3.13. The wire missing the second Cy3.5 dye, 12-0_23456789-12 was also observed to have a relatively high transfer efficiency. The other wires given the experimental measurement error are all about equivalent. The completed set the averaged steady-state fluorescence spectra can be found in Section 3.8.10.

![Graph](image)

Figure 3.8. The experimentally determined WTE, $E_{ae}$, and $E_{ee}$ for wires with a single Cy3.5 dye skips. The WTE, $E_{ae}$, and $E_{ee}$ are shown as green, blue, and red bars, respectively.

The homoFRET region was discretely increased in length in the second set of wires as can be seen in Figure 3.9. The first sign of measurable energy transfer begins when the number of Cy3.5 dye repeats reaches five dyes. The addition of the eight Cy3.5 results in a sizable increase in the energy transfer.
Figure 3.9. The experimentally determined WTE, $E_{ae}$, and $E_{ee}$ for wires with as the number of Cy3.5 repeats are extended from one to ten. The WTE, $E_{ae}$, and $E_{ee}$ are shown as green, blue, and red bars, respectively.

The third and fourth sets of wire test various homoFRET combinations including skipping alternating dyes, skipping dyes in sets of two and three as is shown in Figures 3.10 and 3.11.

Figure 3.10. The experimentally determined WTE, $E_{ae}$, and $E_{ee}$ for wires with varying number of Cy3.5 dye skips. The WTE, $E_{ae}$, and $E_{ee}$ are shown as green, blue, and red bars, respectively.
Figure 3.11. The experimentally determined WTE, $E_{ae}$, and $E_{ee}$ for wires with a single Cy3.5 dye skips. The WTE, $E_{ae}$, and $E_{ee}$ are shown as green, blue, and red bars, respectively.

3.4.6 Monte Carlo Simulations

The Monte Carlo experiments predicted faster FRET decay rates in efficiency than were observed in the experiment. The Monte Carlo simulations results for the full wire with a randomly missing Cy3.5 dye are shown in Figure 3.12. The ideal device curve is depicted as a dashed line and assumes all the dyes are attached to the DNA template. The non-ideal curve is shown as a solid line assumes some of the dyes have randomly detached from the DNA template and are free in solution. The formation yield was determined by fitting the full structure $E_{ae}$ from Figure 3.12 to the experimentally determined energy transfer values.
Figure 3.12. (a) The geometry of the simulated wire used in the Monte Carlo FRET simulations. The dyes are free to randomly move around their attachment point. (b) A plot of the simulated $E_{ae}$ for the full wires with a set number of Cy3.5 dyes missing. The model assuming 100% yield and 75% yield are shown in red and green, respectively. The experimentally $E_{ae}$ values are shown with green squares. The model generally fits the wire’s experimentally determined efficiency when a 75% formation yield was assumed for the wires.

Figure 3.13. The experimentally determined WTE, $E_{ae}$, and $E_{ee}$, for the wires with single Cy3.5 dye skips, that exhibited the highest transfer efficiencies. The WTE, $E_{ae}$, and $E_{ee}$ are shown as green blue and red bars, respectively. The wire that was missing the first Cy3.5 was found to have the highest WTE, $E_{ae}$, and $E_{ee}$.
Monte Carlo Simulation vs. Experiment

Figure 3.14. A plot of the simulated $E_{ae}$ and $E_{ee}$ for the full wire with one missing Cy3.5 dye. All ten possible variations are shown. The experimentally collected data for $E_{ae}$ and $E_{ee}$ are shown as blue and red squares, respectively. The efficiency was modeled assuming 75% formation yield.

3.5 Discussion

The investigation found that the photonic wire’s transfer efficiency for all of the homo-FRET configurations evaluated was noticeably lower than predicted values. The main contributing factors likely causing the reduced performance include: (1) difference between designed and actual fluorophore spacing, (2) fluorescent quenching by DNA, (3) formation yield, and (4) only exciting a small fraction of the wires during the steady-state fluorescence characterization. The investigation also found that many distinct configurations of photonic wires resulted in similar transfer efficiency. However, the spacing between the first and last fluorophores of the homo-FRET section seems to be the single most important factor in determining the wire-transfer efficiency.
The first factor that likely lead to the reduced transfer efficiency was that the fluorophore spacing in the formed wire was found to be larger than the designed spacing. The original wire design assumed that the fluorophores would naturally orient in the center of the six-helix bundle, due to the steric hindrance and the length of their attachment linkers. However, experimental evidence suggests that, on average, the fluorophores are likely located closer to their attachment locations and are not in the center of the six-helix bundle. When the dyes were assumed to orient in the center of the six-helix bundle, they were equally spaced, with a 6-bp (2-nm) separation. However, when the fluorophores orient closer to their attachment points, they are not equally spaced. The difference in spacing results from the first Cy3.5 fluorophore being attached to helix 3, whereas the second Cy3.5 is on helix 2, as can be seen in Figure 3.1.

The spacing discrepancy is a result of placing the second AF488 on helix 1. When the first Cy3.5 is removed, the distance between the AF488 and the second Cy3.5 is much less than twice the distance of the first Cy3.5. The distances between the last AF488 and the first and second Cy3.5 binding sites were calculated to be 3.3 and 4.6 nm. The first observation is that 4.6 nm is more than double the distance of 2 nm, our designed spacing. The discrepancy results from the fluorophore’s linker (1 nm long) being assumed to work in favor of the design. However, since the Cy3.5 fluorophores were labeled with double phosphoramidites, which have been shown to position the fluorophores closer to the DNA than the succinimidy1 ester linkers used for the AF488 and AF647 fluorophores, the actual dye distances were larger than the original wire design. The fluorophore spacing seems to be supported by the
measured donor-acceptor pair FRET efficiencies. The wire missing the first Cy3.5 fluorophore was found to have the highest transfer efficiency. The FRET pair transfer efficiency from the two donor AF488 fluorophores to the first Cy3.5 was found to be 49.3%, whereas the transfer efficiency to the second Cy3.5 was found to be 40.7%, only an 8.6% decrease.

The second factor is fluorophore quenching caused by guanine and cytosine nucleotides in the scaffold and staples. The DNA sequences guanine and cytosine are known to cause fluorophore quenching especially if the fluorophore is in proximity. Because we have little control over the DNA sequence of the scaffold strand, it is nearly impossible to ensure that guanine and cytosine nucleotides are not located near the fluorophore attachment position and/or between successive fluorophores. One possible solution could be to use a scaffold composed of only adenine and thymine nucleotides. Examining the scaffold sequence near the fluorophore binding location revealed that there are cytosine and guanine fluorophores near the binding location of the fluorophores. However, they are relatively randomly distributed and thus should not quench any fluorophore. They do, however, likely lead to an overall reduction in wire-transfer efficiency because of their collective quenching throughout the entire wire.

The third factor is related to the formation efficiency. The origami formation yield was high, >90%, as determined by gel electrophoresis. The Monte Carlo simulations best fit the measured donor-acceptor pair FRET efficiencies when a 75% formation yield was assumed. The simulations assumed that dyes not attached to the origami were free floating in solution while the origami formation yield only
examined the formation of the DNA template without dyes attached. These findings suggest that the photonic wire’s formation yield, with fluorophores attached to the origami, was lower and around 75%. A few possible explanations for variations between the calculated and measured transfer efficiency are, unfavorable dye orientations and the model did not account for contributions from partial structures were not considered. Gel electrophoresis of the Amicon purification procedure, Section 3.8.8, revealed that nearly all unincorporated labeled and unlabeled staple strands were removed in the third round of Amicon purification. A possible explanation for this finding is that, although the origami formed in high yield, some of the fluorophores’ attachments sites may not have been available, meaning that the binding site was not properly formed and thus prevented the labeled DNA from binding or that the binding site was poisoned by an unlabeled staple strand whose sequence was complementary to the binding sites. Previous statistical analysis of attaching DNA functionalized gold nanoparticles to origami found that even when four DNA tethers were used for attachment of single particles, the highest attachment yield achievable was 97%.5

The fourth and final factor that will be discussed is related to the characterization technique. Calculations revealed that only a small fraction (around 1 in 20) of the devices were being excited in the steady-state fluorescence at any given time. The wire-transfer efficiency was developed as a way of overcoming the characterization limitation because it is a measure of the transfer efficiency that is independent of the number of photons put into the wire.
3.6 Conclusion

In conclusion, we designed built and characterized nanoscale photonic wires with a reconfigurable homoFRET region. Over 50 different homoFRET configurations were experimentally characterized and mathematically modeled using Monte Carlo simulations. The investigation demonstrated that the energy transfer efficiency is not dependent on the number of homoFRET dye repeats but rather on their geometric configuration. The wires overall energy transfer efficiency was limited by dye attachment yield and lack of control over the individual fluorophores dipole orientations. If the full potential of photonic wires is to be realized for nanoscale communications and advanced biosensing applications, new fluorophore labeling techniques must be investigated and developed.

3.7 Acknowledgments

The first author acknowledges the Pathways Internship Program at the Naval Research Laboratory that helped make this work possible. The authors would also like to thank Dr. Reza Zadegan, a post-doctoral fellow in Nanoscale Materials and Device Group, at Boise State University, for helping prepare the DNA scaffold used for the project. We also acknowledge the Office of Naval Research and the NRL Nanosciences Institute. W.R.A. is grateful to NSERC, S.A.D., and ASEE post-doctoral fellowships through NRL. The authors also recognize the NRL Nanosciences Institute and DTRA JSTO MIPR # B112582M.
3.8 Supporting Information

3.8.1 Quantum Yields Measurements

The fluorescence quantum yields were experimentally determined using Equation (3.11).\(^{19}\) Where \(\Phi_{\text{standard}}\) and \(\Phi_x\) are the fluorescence quantum yields of the standard and the unknown sample, \(m_x\) and \(m_{\text{standard}}\) are the slopes from the intensity vs. absorbance plot and \(n_x\) and \(n_{\text{standard}}\) are the refractive index of the solvents for the unknown sample and for the standard.

\[
\Phi_x = \Phi_{\text{standard}} \left( \frac{m_x}{m_{\text{standard}}} \right) \left( \frac{n_x^2}{n_{\text{standard}}^2} \right)
\]  

(3.11)

![Figure 3.15](image-url)  

Figure 3.15. Plots of the fluorescence intensity versus absorbance for the DNA labeled AF488 fluorophores attached to the origami and for the fluorescent standard Fluorescein used to determine the quantum yield of the two DNA labeled AF488 fluorophores.
Figure 3.16. Plots of the fluorescence intensity versus absorbance for the DNA labeled Cy3.5 fluorophores attached to the origami and for the fluorescent standard Rhodamine 640.

Figure 3.17. Plots of the fluorescence intensity versus absorbance for the DNA labeled AF647 fluorophores attached to the origami and for the fluorescent standard Rhodamine 800.
3.8.2 Spectral Decomposition

A|E UV-Vis-IR spectral software a freeware Matlab® program was used to decompose the collected spectra into their spectral components. The program used a least-squares fitting algorithm to fine the linear combination of spectra components which best fit the collected spectra. An example is shown in Figure 3.18 and Figure 3.19. The approach described above follows the analysis of FRET-based DNA photonic wires previously reported.24,31

Figure 3.18. The reference steady-state fluorescence spectra for AF488, Cy3.5, and AF647, input to the A|E UV-Vis-IR Spectral Software. These reference spectra were used by the program to find the least-squares fits and generate the individual spectra components
Figure 3.19. A plot depicting the spectra decomposition process for the full photonic wire consisting of AF488, Cy3.5, and AF647 fluorophore.

Figure 3.20. A plot depicting the spectra decomposition process for a photonic wire consisting of Cy3.5, and AF647
3.8.3 M13mp18 Cut Preparation

The scaffold for the DNA origami wire was prepared by cleaving the M13mp18 plasmid with two restriction enzymes, EcoRI and BglII, to create a 704 nucleotide long scaffold. To create a the double-stranded region on M13mp18 plasmid required for the restriction enzymes two, 36 basepair ssDNA oligos,

**EcoR1 Seq** GCT CGA ATT CGT AAT CAT GGT CAT AGC TGT TTC CTG

**BglII Seq** CCG GAG AGG GTA GCT ATT TTT GAG AGA TCT ACA AAG,

complementary to the EcoRI and BglII, cleaving locations respectively, were added to the stock M13mp18. The salt adjusted melting temperature for these strands was 74.2 °C. 64 µL (10 µM) of the two 36 basepair ssDNA oligos were added to 800 µL stock M13mp18 (concentration 360 nM). 18.6 µL MgCl₂ (1000 mM) is added to increase the affinity for binding. 10 mM excess was added to overcome the EDTA present in the 10× TAE buffer. 20 mM MgCl₂ final. The primer strands are added to the M13 in a two to one excess primers to M13. The solution was then split into 200 µL Lo Bind centrifuge tubes, 100 µL of solution in each was then annealed using the following program: 90 °C 1 minute, 90 °C -1.0 °C per 5 seconds 10x, 80 °C -1.0 °C per 15 seconds 15x, 65 °C -1.0 °C per 30 seconds 45x, hold 4 °C.

After the anneal was complete. The samples were combined in a 1.5 µL centrifuge tube. 95 µL of 10x Fast Digest Buffer was added followed by 16 µL of each enzyme. The sample was again split into 200 µL Lo Bind centrifuge tubes and annealed at 37 °C for two hours and then 85 °C for 10 minutes to denature the enzyme. The short 704 bp cut was separated from the long M13 ssDNA in a 1.5%
agarose gel using agarose gel electrophoresis. The DNA was extracted from the gel by placing the gel containing the DNA on top of a TLC plate and exciting the gel with a UV lamp. The DNA absorbed the UV light and a shadow appears on the TLC plate indicating the location of the DNA in the gel. The TLC plate approach allowed for the removal of the DNA from the gel without the use of an intercalating dye which could interfere with optical measurements. The properties of the M13 cut are as follows: M13 Cut ssDNA Molecular Wt. = 216,917.5 g mole, 1.0 O.D. = 135 nM, 1mL = 29.3 µg.

The resulting gel was illuminated with UV light on a TLC plate. Two bands were observed and the lower band containing the M13 cut was extracted from the agarose gel using Freeze and Squeeze tubes. The gel containing the M13 cut was cut up using a razer blade and then placed in the tube. The tube was then spun at 17,000 rcf for 3 minutes. In order to increase the yield for the extraction after the first spin 100 µL of 1×TAE was then added the gel and stirred. The tube was spun again three more times adding 100 µL of 1×TAE each time. The concentration of the M13 Cut was determined the Biophotometer.
3.8.4  **M13 Cut Sequence**

(5' → 3') TCG AGC TCG GTA CCC GGG GAT CCT CTA GAG TCG ACC TGC AGG CAT GCA
AGC TTG GCA CTG GCC GTC GTT TTA CAA CGT CGT GAC TGG GAA AAC CCT GGC GTT
ACC CAA CTT AAT CGC CTT GCA GCA CAT CCC CCT TTC GCC AGC TGG CGT AAT AGC
GAA GAG GCC CGC ACC GAT CGC CCT TCC CAA CAG TTG CGC AGC TGG CTG AAT GGC GAA
TGG CGC TTT GCC TGG TTT CCC GCA CCA GAA GCC GTG CCC GAA AGC TGG CTG GAG
TGC GAT CTT CCT GAG GCC GAT ACG GTC GTC GTC CCC TCA AAC TGG CAG ATG CAC
GGT TAC GAT GCG CCC ATC TAC ACC AAC GTA ACC TAT CCC ATT ACG GTC AAT CCG
CCG TTT GTC CCC ACG GAG AAT CCG ACG GGT TGT TAC TCG CTC ACA TTT AAT GTT
GAT GAA AGC TGG CTA CAG GAA GCC CAG ACG CTA ATT ATT TTT GAT GGC GTT CCT
ATT GGT TAA AAA ATG AGC TGA TTT AAC AAA AAT TTA ACG CTA ATT TTA ACA AAA
TAT TAA CGT TTA CAA TTT AAA TAT TTG CTT ATA CAA TCT TCC TGT TTT TGG GGC TTT
TCT GAT TAT CAA CCG GGG TAC ATA TGA TTG ACA TGC TAG TTT TAC GAT TAC CGT TCA
TCG ATT CTC TTG TTT GCT CCA GAC TCT CAG GCA ATG ACC TGA TAG CCT TTG TA

3.8.5  **Scaffold Fill Sequence**

(5' → 3') ATG CTG ATC AA
Table 3.3. Unlabeled strands for six-helix bundle nanotube.

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<th>bps</th>
<th>Mp.1,2</th>
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</thead>
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<tr>
<td>0 [19]-1 [26]</td>
<td>ACAGGAAGATTGATTTAATTTGTAACGTTAATATT</td>
<td>37</td>
<td>65.8</td>
</tr>
<tr>
<td>1 [110]-0 [117]</td>
<td>TCTAGAGATCCCGGTGACCTACGTCATTGCT</td>
<td>35</td>
<td>78.8</td>
</tr>
<tr>
<td>1 [27]-0 [33]</td>
<td>TTGTAAAGTCCGATTTCCTCCCGGTGATTTGATAATCAG</td>
<td>34</td>
<td>71.6</td>
</tr>
<tr>
<td>1 [48]-1 [68]</td>
<td>TGGGAACAAAGCCGGAGTGA</td>
<td>21</td>
<td>61.2</td>
</tr>
<tr>
<td>1 [70]-0 [75]</td>
<td>CGTAATGGAATGTTTAACCGGTAACGTTAATCGT</td>
<td>33</td>
<td>72.5</td>
</tr>
<tr>
<td>1 [90]-1 [109]</td>
<td>TGGTCTAGCTGCAAGCTGAC</td>
<td>20</td>
<td>62.5</td>
</tr>
<tr>
<td>2 [104]-2 [84]</td>
<td>TGCATGCAATGGCGCCTGTA</td>
<td>21</td>
<td>63.2</td>
</tr>
<tr>
<td>2 [20]-4 [14]</td>
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</tr>
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<td>2 [41]-2 [21]</td>
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</tr>
<tr>
<td>2 [62]-2 [42]</td>
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<td>21</td>
<td>53.4</td>
</tr>
<tr>
<td>2 [83]-2 [63]</td>
<td>ACCGTGACCTGCCTTTCATC</td>
<td>21</td>
<td>62.2</td>
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<td>CTTCGCATATTCCGACCTTGATACCC</td>
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<td>69.5</td>
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1 Calculated melting point (Mp) of ssDNA strands. Oligonucleotide Properties Calculator.
2 The salt adjusted melting temperature was used for all Mp calculations.
Table 3.4. Labeled strands for six-helix bundle nanotube.

<table>
<thead>
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<th>Name</th>
<th>Sequence (5′ → 3′)</th>
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<th>Mp.</th>
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<td>71.6</td>
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<td>69.1</td>
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<td>1[48]-1[68]</td>
<td>Cy3.5-TGGGAAACAAAACCGGCCTTGGA</td>
<td>21</td>
<td>61.2</td>
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<tr>
<td>1[70]-0[75]</td>
<td>Cy3.5-CTTAATGGGATAGGTTACCTCAGGTCATTGCTA</td>
<td>33</td>
<td>72.5</td>
</tr>
<tr>
<td>1[90]-1[109]</td>
<td>Cy3.5-TGGTGTAGCTGCAGGTCGAC</td>
<td>20</td>
<td>62.5</td>
</tr>
<tr>
<td>2[104]-2[4]</td>
<td>647-TGCATGCATGGGCACTTCG</td>
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<tr>
<td>2[20]-4[14]</td>
<td>AF488-TTGGTTAAATTTAACCATAAGGGCACTTGGGG</td>
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<td>69.4</td>
</tr>
<tr>
<td>2[41]-2[21]</td>
<td>Cy3.5-AACATTAATGTAGCGAATG</td>
<td>21</td>
<td>55.4</td>
</tr>
<tr>
<td>2[62]-2[42]</td>
<td>Cy3.5-AACATTAATGTAGCGAATG</td>
<td>21</td>
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<tr>
<td>2[83]-2[63]</td>
<td>Cy3.5-ACCGTAGCATCTCTATTTCAT</td>
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</tbody>
</table>

1 Calculated melting point (Mp) of ssDNA strands. Oligonucleotide Properties Calculator.

2 The salt adjusted melting temperature was used for all Mp calculations.
3.8.6 CanDo Analysis

The DNA origami’s scaffold routing and staple crossover design was analyzed using finite-element-based modeling in CanDo to predict the 3D solution shape and flexibility of the folded wire and to optimize the DNA scaffold and staple routing design. The modeling results for the final scaffold routing and staple over crossover design are shown in Figure 3.21. In the end, the wire’s structural rigidity had to be balanced with the fluorophores binding locations which assumed a high priority.

![CanDo Analysis](image)

Figure 3.21. The Cando modeling results for the photonic wire. The blue and red shading indicate areas of low and high structural flexibility, respectively.

3.8.7 DNA Origami Anneal Program

Table 3.5. DNA anneal program used to fold the DNA origami photonic wire. In order to reduce the likelihood of dye degradation the time spent at elevated temperatures above 77 °C was minimized. The anneal program was developed by analyzing the melting curve for the origami wire.

<table>
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<th>Temp 2 (°C)</th>
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<td>77</td>
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</tr>
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</table>
3.8.8 DNA Origami Purification

To remove the excess staple strands from the formed origami wire 50k Amicon centrifuge filters were used. The 1×TAE with 15 mM MgCl$_2$ buffer and samples were cooled in ice bath for approximately 5 to 10 minutes. The sample was cool down to make the DNA origami wire more robust and less likely to decompose during the purification process. The Amicon filters were rinsed with 1×TAE with 15 mM MgCl$_2$ prior to introducing the origami to remove the glycerol protective coating from the Amicon membrane as is recommend by the manufacturer. During the rinse step the filters were centrifuged at 14,000 rcf for 1 minute. The filters were then inverted and spun again to remove any excess buffer remaining in the bottom of the filter. Next, 380 µL of chilled 1×TAE with 15 mM MgCl$_2$ buffer was placed in each Amicon filter along with 120 µL of unpurified origami solution. The filters were centrifuged for 3 minutes at 7,000 rcf. The rinse procedure was repeated three times discarding the buffer containing the excess staples and adding 400 µL of 1×TAE with 15 mM MgCl$_2$ each time. Gel electrophoresis confirmed that three spin steps were sufficient to remove all access DNA labeled fluorophores from solution. The gel is shown in Figure 3.22.
Figure 3.22. 1% agarose gel of the formed wire after one, two and three buffer rinses during the Amicon purification process to remove excess staples. No excess staple band appeared after three spin steps.

After the third centrifugation the Amicon filter containing the purified origami was inverted and placed in a new centrifuge vial and spun at 3,500 rcf for 1 minute to extract the purified origami from the Amicon filter. The purified origami was then diluted with 65 µL of 1xTAE with 15 mM MgCl₂ to ensure the sample volume was adequate for the plate reader. 100 µL of each sample was then added to a 96 well plate to conduct the steady-state fluorescence measurement.

3.8.9 Fluorescence Lifetime Measurements Equations

For the lifetimes determinations a biexponential decay function which accounts for two lifetimes and an intensity average, Equation (3.12) was used to fit the spectra.

\[ y(t) = a_1 e^{-t/\tau_1} + a_2 e^{-t/\tau_2} \]  (3.12)
where, $a_1$ and $a_2$ are fitting parameters and $\tau_1$ and $\tau_2$ are the lifetimes in units of ns.

Equation (3.13), a double exponential decay equation was used to fit the fluorescence lifetime anisotropies spectra. The double exponential decay equation takes into account the movement of the molecule with the linker and the tumbling of the entire DNA device.

$$y(t) = a_1 e^{-t/\tau_1} + a_2 e^{-t/\tau_2} + y_0$$ (3.13)

where, $a_1$ and $a_2$ are fitting parameters, $\tau_1$ and $\tau_2$ are interpreted as the rotational correlation times, and $y_0$ is the residual anisotropy.

The average lifetime was found using Equation (3.14).\textsuperscript{21}

$$\tau_{\text{Avg}} = \frac{\alpha_1 \tau_1^2 + \alpha_2 \tau_2^2}{\alpha_1 \tau_1 + \alpha_2 \tau_2}$$ (3.14)

where, $\alpha_1$ and $\alpha_2$ are the preexponential terms which depend on the fluorophores concentration, absorption, and quantum yield.\textsuperscript{20}

### Table 3.6. The experimentally determined fluorophore lifetimes for several photonic wires.

<table>
<thead>
<tr>
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<th>89-12</th>
<th>9-12</th>
<th>9-\textsuperscript{9}</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\tau_1$ (ns)</td>
<td>0.54 ± 0.01</td>
<td>0.47 ± 0.01</td>
<td>1.08 ± 0.06</td>
</tr>
<tr>
<td>$\tau_2$ (ns)</td>
<td>1.87 ± 0.03</td>
<td>1.87 ± 0.04</td>
<td>2.99 ± 0.36</td>
</tr>
<tr>
<td>Avg. Lifetime (ns)</td>
<td>0.84 ± 0.05</td>
<td>0.68 ± 0.05</td>
<td>1.66 ± 0.23</td>
</tr>
<tr>
<td>Adj. R-Square</td>
<td>0.9995</td>
<td>0.9991</td>
<td>0.9991</td>
</tr>
</tbody>
</table>
Figure 3.23. Time-dependent fluorescent decay curves used to investigate FRET transfer between the Cy3.5 and AF647 fluorophores. The fluorescence lifetime of Cy3.5 was reduced in the presence of AF647.

Table 3.7. The experimentally determined fluorophore lifetimes for several Cy3 homoFRET wire configurations.

<table>
<thead>
<tr>
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<th>5</th>
<th>45</th>
<th>456</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\tau_1) (ns)</td>
<td>1.01 ± 0.14</td>
<td>0.81 ± 0.07</td>
<td>1.40 ± 0.09</td>
</tr>
<tr>
<td>(\tau_2) (ns)</td>
<td>2.5 ± 0.2</td>
<td>2.16 ± 0.1</td>
<td>3.9 ± 2.4</td>
</tr>
<tr>
<td>Avg. Lifetime (ns)</td>
<td>1.82 ± 0.29</td>
<td>1.51 ± 0.15</td>
<td>1.74 ± 0.13</td>
</tr>
<tr>
<td>Adj. R-Square</td>
<td>0.9987</td>
<td>0.9991</td>
<td>0.9991</td>
</tr>
</tbody>
</table>

Figure 3.24. Time-dependent fluorescent decay curves used to investigate FRET transfer in the wire’s Cy3.5 homoFRET region.
3.8.10 Emission and Excitation Spectra

Figure 3.25. (a-b) Steady-state fluorescence emission spectra collected by exciting the wire with 466 nm and 585 nm light, respectively. (c) Steady-state fluorescence excitation spectra to determine how the wires fluorescence intensity at 700 nm changes as the excitation wavelength is varied from 450 nm to 700 nm.
Figure 3.26. (a-b) Steady-state fluorescence emission spectra collected by exciting at 466 nm and 585 nm, respectively.
Figure 3.27.  (a-b) Steady-state fluorescence emission spectra collected by exciting the wire with 466 nm and 585 nm light, respectively.  (c) Steady-state fluorescence excitation spectra to determine how the wires fluorescence intensity at 700 nm changes as the excitation wavelength is varied from 450 nm to 700 nm.
Figure 3.28. (a-b) Steady-state fluorescence emission spectra collected by exciting the wire with 466 nm and 585 nm light, respectively. (c) Steady-state fluorescence excitation spectra to determine how the wires fluorescence intensity at 700 nm changes as the excitation wavelength is varied from 450 nm to 700 nm.
REFERENCES


CHAPTER FOUR: FRET-BASED LIGHT HARVESTING DEVICES

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4.1 Abstract

Photonic devices that exploit Förster Resonance Energy Transfer (FRET) to direct photonic energy are of great interest due to their potential applications in light harvesting, biocomputing, and biosensing. While such systems have been demonstrated, additional work is required to elicit a set of design rules that will allow for the construction of more efficient light harvesting devices. In particular, homogeneous FRET (homoFRET) in complex antenna systems is not well understood. In this work, we designed, constructed, and characterized DNA self-assembled, multi-fluorophore, light-harvesting devices that have extendable homoFRET regions, which we showed increase the device’s antenna gain. The devices were created by arranging fluorophores in three different geometries. The geometries evaluated were a four-arm star, an eight-arm star, and a dendrimer structure. Each device incorporated an extendable homoFRET region that could be extended for up to six Cy3 repeats. Steady-state and time-resolved fluorescence measurements were used to characterize the devices. A nearly six-fold antenna gain was observed in the dendrimer device. Additionally, we showed that an energy transfer efficiency of 3.0 ± 1.2% is possible through eight FRET steps (four heterogeneous and four homogeneous). The findings demonstrate that homoFRET can be used to increase the energy harvesting capability of photonic devices.

4.2 Introduction

DNA self-assembly is a robust method for directing the assembly of nanoscale devices.\textsuperscript{1,2} The specificity of the complementary Watson-Crick base pair
hybridization allows for functionalized elements, such as quantum dots, gold particles, and/or fluorophores, to be attached with nanoscale precision at specific locations on DNA templates. The versatility of this approach allows for the creation of complex multidimensional shapes. The accessibility of commercially available synthetic DNA, along with a variety of functionalized nanoparticles and labeled fluorophores, reinforces the technique’s versatility and availability. Open access design tools, such as Cadnano, vHelix, and NanoEngineer, allow for the relatively rapid design of structural DNA templates. With the above considerations in mind, it is easy to see why DNA self-assembly is an attractive technique for the construction of photonic devices.

Over the last decade, researchers have demonstrated the successful construction of many nano-sized devices, ranging from biosensors to energy harvesting devices. Energy harvesting devices usually exploit FRET to transfer energy. FRET is of considerable interest due to the high transfer efficiency observed in plant photosynthesis. FRET is the nonradiative transfer of energy between oscillating dipoles of fluorescent molecules known as fluorophores. Heterogeneous FRET (heteroFRET), the energy transfer between different molecules is typically used in energy harvesting because the energy is transferred in a downhill, cascade-like manner. Recent work has shown that homoFRET, the transfer of energy between the same fluorophores, is an alternative method that can be used in combination with heteroFRET to increase the transfer distance. HomoFRET, in theory, results in no energy loss when the distances between the donor and acceptor are less than about 0.4 times the Förster distance. The downside to homoFRET is that control over
energy transfer directionality is lost due to the random walk nature of the process.\textsuperscript{16}

The Albinsson Group was the first to demonstrate the advantages of adding a homoFRET region to act as a bridge between a donor and an acceptor molecule.\textsuperscript{17} Expanding on these ideas, S. Diaz \textit{et al.} created three and five-dye molecular photonic wires that incorporated a six-dye homoFRET region. Their wire was capable of transferring energy over 30 nm with a 3\% efficiency.\textsuperscript{18} The losses were attributed to DNA formation deficiencies, incompatible static dye orientations, DNA-dye interactions, and energy sinks, which have been observed by others.\textsuperscript{4,19}

Past investigations found that 5-dye systems composed of fluorophores arranged in three geometries (a four-arm star, and eight-arm star, and a dendrimer geometry) resulted in relatively high-energy transfer efficiencies.\textsuperscript{15} The fluorophores in these systems were spaced at half the Förster radius (0.5 $\times$ $R_0$). The goal of this investigation was to determine whether photonic devices with homoFRET regions could be created using the same geometries previously investigated. Could the principles governing homoFRET be utilized to increase the devices’ light-harvesting abilities and potentially extend the energy transfer distance? With this question in mind, we designed, synthesized, and characterized three devices. Each 5-dye device incorporated an extendable (up to six dyes) homoFRET region. Alexa Fluor 488 (AF488), Cy3, Cy3.5, Cy5, and Cy5.5 were the five fluorophores chosen for the devices.

The absorption and emission spectra for the fluorophores are shown in Figures 4.1a and 4.1b, respectively. The emission spectra have been normalized by the quantum yield (QY) of each respective dye. The overlap integral for the
donor-acceptor pairs is shown in Figure 4.1c. The overlap integral is a measure of the degree of spectral overlap between donor-acceptor pairs and depends on both the absorption spectrum of the acceptor and the emission spectrum of the donor. The figures show that the possibility exists to transfer energy over a relatively wide (over 220 nm) wavelength range. The AF488 fluorophore can be directly excited with light of a wavelength around 475 nm. The energy is then transferred to the terminal Cy5.5 fluorophore where it is re-emitted as 700 nm wavelength light. The fluorophores act collectively to absorb light throughout a significant portion of the visible spectrum that enables effective light harvesting.

![Absorption Spectra and Emission Spectra](image)

**Figure 4.1.** Fluorophore photophysical properties. (a) A plot of the fluorophore extinction coefficients. (b) The plot of the fluorescence emission spectra normalized to the quantum yield of each dye. (c) The integrand of the overlap integral as a function of wavelength for the indicated donor-acceptor combinations.
4.3 Methods

4.3.1 Materials

The labeled and unlabeled DNA strands were obtained from Integrated DNA Technologies (Coralville, USA) and Eurofins Operon (Huntsville, USA). The melting temperatures and sequences can be found in the Section 4.9.1. All dye labeling was performed on terminal bases, either succinimidyl ester labeling with six carbon linker for AF488 or double phosphoramidite labeling with a three carbon linker for the cyanine dyes (Cy3, Cy3.5, Cy5, and Cy5.5). The quantum yield of each dye was experimentally determined using fluorescent standards. Fluorescein, Cresyl Violet, Rhodamine 640 (Rhodamine 101), Rhodamine 6G, and Rhodamine 800 (LD 800) were used as the standards to compare the respective fluorophores, AF488, Cy3, Cy3.5, Cy5, and Cy5.5. To account for variation in the QY measurements an average of several independent QY measurements were made to determine the value used in our models.

4.3.2 Design

Each of the devices consisted of two centrally located Cy5.5 fluorophores in addition to Cy5, Cy3.5, Cy3, and AF488 fluorophores moving outwards from the center. Each device incorporated an extendable one to six Cy3 fluorophores homoFRET repeat region between the Cy3.5 and AF488 fluorophores. To describe the Cy3 homoFRET regions, we adopted a notation describing the number of Cy3 repeats in each device as R1, R2, R3 ... R6, designating the number of Cy3 fluorophores in each arm. The R1 device contains one Cy3 chromophore, whereas the R6 device contains six Cy3 fluorophores. The device functions by adsorbing light initially
though the AF488 fluorophores from which it is then transferred through non-radiative FRET to the terminal Cy5.5 fluorophores where it is readmitted. The designed donor-acceptor distance $r_{DA}$ in each device was set at $0.5 \times R_0$. The four-arm star and eight-arm star devices were comprised of four and eight linear arms, respectively. The dendrimer device incorporated a 2-to-1 branching ratio for each of its branches, meaning that the ratio of donor to acceptor molecules is 2-to-1. The exception is the Cy3 homoFRET region, which is comprised of a linear arrangement so that all the devices homoFRET regions were the same.

The following naming conventions were adopted. AF488-Cy5.5 represents a device in which all dyes are present. Cy3-Cy5.5 represents a device with an unlabeled DNA strand in place of the original AF488 dye strand. The four-arm and eight-arm stars are shown in Figures 4.2a and 4.2b. The location of the stars represents the fluorophores’ attachment locations to the DNA oligos. The individual arms consist of three unique DNA strands. The backbone of each arm is a single unlabeled strand that incorporates three sequence regions, one that is complementary to the AF488 labels strand, a repeating nine base pair region for the Cy3 labeled strand, and a nine base pair region to attach to the core of the device. The schematics for the dendrimer are shown in Figure 4.2c. Complete design diagrams depicting the exact location of the fluorophores are in the Section 4.9.2.
Figure 4.2. Schematics of the three DNA self-assembled light harvesting devices. For simplicity, the DNA has been represented as parallel lines. The red, orange, yellow, green, and blue stars represent the Cy5.5, Cy5, Cy3.5, Cy3, and AF488 fluorophores attachment locations, respectively. The devices each have an extendable Cy3 homoFRET region that was utilized to create structures of increasing arm length. (a-c) The cores of the four-arm star, eight-arm star, and dendrimer devices, respectively. The cores consist of two centrally located Cy5.5 fluorophores in addition to Cy5, Cy3.5, and Cy3 fluorophores moving outwards from the center. The dendrimer is the only device to contain Cy3 fluorophores in the core. (d-e) The individual star arms and dendrimer arms R1-R6, respectively. The R1 device contains one Cy3 chromophore whereas the R6 device contains six Cy3 fluorophores. (f-h) The full four-arm star R2, the eight-arm star R2, and the dendrimer R2 devices, respectively. For clarity in the 2D representation, two of the dendrimer arms were extended. Missing arms are noted as black zig-zags. Black dotted oval circles represent individual arm attachment locations.
4.3.3 Assembly

Devices were prepared in 2.5x PBS (phosphate buffered saline: \(1x = 137 \text{ mM NaCl, 10 mM phosphate, 2.7 mM KCl, (pH 7.4)}\)). PBS buffer without Mg\(^{2+}\) was used to prevent quenching from high ionic concentrations.\(^{22,23}\) Final DNA device concentration was set at 0.35 \(\mu\text{M}\). The samples were annealed in a PCR cycler by heating to 94 °C, held for four minutes, then the temperature was decreased by 1 °C per minute until the final temperature reaches 4 °C. The devices were analyzed without any additional post-assembly purification. The salt adjusted melting temperature for each strand was estimated using Oligonucleotide Properties Calculator.\(^{24}\) The shortest nine nucleotide strand’s estimated melting temperature was 28 °C whereas the longest DNA strand had an estimated melting temperature of 89 °C.

The samples were stored in the dark at 4 °C to minimize the effects from photobleaching. Extended annealing protocols were also evaluated, but they were found to provide no observable formation improvement. Agarose gel electrophoresis was used to assess the devices formation yield. 10 \(\mu\text{L}\) of each device (20 \(\mu\text{M}\)) was loaded into the respective wells R1 through R6. The gels are shown in Figure 4.3. Three percent agarose gels were stained with GelRed (Biotium, Hayward, USA). The formation yield for each device was estimated by analyzing the intensity of the stained gel bands in each lane.

4.3.4 Steady-State Absorption and Fluorescence Measurements

The devices steady-state absorbance and fluorescence measurements were collected to assess the transfer efficiency and antenna gain. The absorption and
fluorescence spectra of the individual fluorophores were measured and used to calculate the overlap integrals and Förster radius for each fluorophore. The calculated values can be found in Table 4.1. The absorption spectra were collected using an Agilent 8453 diode array UV-Vis spectrometer. In comparison, the steady-state fluorescence spectra were collected using a Tecan Infinite M1000 dual monochromator (Tecan, Research Triangle Park, USA). The samples were excited at 466, 515, 635, and 685 nm in microtiter 96-well plates. The sample volume was set to 100 µL with a device concentration of 0.20 µM. The fluorescence spectra were collected with a 1 nm step size through a 490-850 nm range at room temperature in 2.5x PBS. The flash frequency was 400 Hz and the integration time 40 µs.

4.3.5 Fluorescence Lifetime and Fluorescence Lifetime Anisotropy

Fluorescence lifetime and fluorescence lifetime anisotropy measurements were collected to understand the energy transfer better and to confirm the presence of homoFRET. A time-correlated single photon counting (TCSPC) technique using a Becker-Hickl SPC-630 board was used. The excitation laser was a 80 MHz, 7 ps pulsed, 532 nm source. The experimental setup is described further in the following citations. The standard fluorescence lifetime measurement was conducted by exciting the samples with a pulse of polarized light. The samples fluorescence was observed through a polarizer set to the magic angle and then filtered through a monochromator. The change in fluorescence intensity of the sample was then recorded. The devices intensity was assumed to decay as the sum of the individual single exponential decays. The lifetimes were extracted by fitting a multi-exponential
decay function to the collected fluorescence decay spectra. The average of the two lifetimes was taken to be the devices overall decay rate. The multi-exponential decay functions are given in Section 4.9.3.

Anisotropy measurements are typically used to reveal the fluorophores average angular displacement or rotational correlation time that result due to the molecules rotational diffusion. However, the method can also be used to detect the presence of homoFRET, which is indicated by a decrease in the samples polarization or anisotropy. The additional decay pathways arising from homoFRET causes the sample’s anisotropy to decrease. The anisotropy measurements were conducted by exciting the sample with a pulse of polarized light. The fluorophores whose dipoles are aligned parallel to the polarization are excited and then at a later time re-emit the light at a different angle. The anisotropy is measured by adjusting the polarizer to parallel and perpendicular alignments.

4.4 FRET Analysis

4.4.1 Overlap Integrals and Förster Distances

The overlap integrals $J$ and Förster distances $R_0$ for the independent dyes were calculated using Equations (4.15) and (4.16), respectively.\textsuperscript{27} Where, $I_D (\lambda)$ is the donor emission spectrum peak area normalized to one, $\epsilon_A (\lambda)$ is the extinction coefficient of the acceptor in units of $M^{-1} cm^{-1}$, and $\lambda$ is the wavelength in units of nm.

$$J (nm^4 M^{-1} cm^{-1}) = \int I_D (\lambda) \epsilon_A (\lambda) \lambda^4 d\lambda \quad (4.15)$$

The Förster distances, $R_0$ were calculated using Equation (4.16) where, n is
the refractive index, set to 1.33 for 2.5x PBS buffer, $\Phi_D$ is the fluorescence quantum yield (QY) of the donor and $\kappa^2$ is the dipole orientation factor, of the dyes, taken to be $2/3$. The $2/3$ value is only valid for fluorophores which are freely rotating dipoles and hence is not entirely valid for our devices. However, the calculated $R_0$ values provide useful distance estimates because the maximum error in dye distance between randomly orientated to parallel dipoles is 35%.$^{27}$

\[
R_0 (nm) = 0.02108 [\kappa^2 \Phi_D n^{-4} J]^{1/6}
\]

(4.16)

### 4.4.2 End-to-End Efficiency

The end-to-end transfer efficiency is a measure of the number of photons emitted by the terminal Cy5.5 fluorophores to the number of the photons introduced at the AF488 fluorophores. The efficiency also accounts for the quantum yield of the donor and final Cy5.5 acceptor fluorophores. Past work$^{17}$ used what we call the anywhere-to-end efficiency, $(E_{ae})$ performance metric, that does not correct for the direct excitation that occurred throughout the devices in the Cy3 homoFRET region. The $E_{ae}$ is defined as Equation (4.17). Where $\phi_{DA}$ is the integrated fluorescence area of the terminal acceptor in the presence of donor, $\phi_A$ is the integrated fluorescence area of the terminal acceptor in the absence of donor, $\phi_D$ is the integrated fluorescence area of the donor in the absence of acceptor, and $\Phi_A$ and $\Phi_D$ are the QY of the terminal acceptor and donor, respectively.

\[
E_{ae} = 100 * \left[ \frac{\phi_{DA} - \phi_A}{\Phi_A} \right] \frac{\phi_D}{\Phi_D}
\]

(4.17)

To account for the indirect excitation of the Cy3, we defined the true
end-to-end efficiency, \( (E_{ee}) \), shown in Equation (4.18). Where \( \phi_{AF488} \) and \( \phi_{Cy5.5} \) are the integrated fluorescence areas of the AF488 donor alone and terminal Cy5.5 acceptor in the presence of all dyes, \( \phi_{Cy3.5 \rightarrow Cy5.5} \) is the integrated fluorescence area of the Cy5.5 terminal acceptor from the Cy3.5-Cy5.5 device, \( \phi_{AF488} \) is the integrated fluorescence area of the donor in the absence of any acceptor, and \( \Phi_{AF488} \) and \( \Phi_{Cy5.5} \) are the QYs of AF488 and Cy5.5, respectively.

\[
E_{ee} = 100 \ast \left[ \frac{\phi_{Cy5.5} - \phi_{Cy3.5 \rightarrow Cy5.5}}{\Phi_{Cy5.5}} \right] / \frac{\phi_{AF488}}{\Phi_{AF488}} \tag{4.18}
\]

Like the \( E_{ae} \), the \( E_{ee} \) represents the percentage of photons emitted by the final acceptor to the number of photons absorbed by the initial input or donor dye however, it corrects for the indirect excitation of the Cy3 fluorophores.\(^{17,28} \) The \( E_{ae} \), in contrast to the \( E_{ee} \) shows the indirect excitation that occurred throughout the devices in the Cy3 homoFRET region. The \( E_{ee} \) corrects for the direct excitation that occurs as a result of the Cy3 fluorophores being partially excited by the 466 nm light, which was used to directly excite the AF488 fluorophores. Plots comparing the \( E_{ae} \) and \( E_{ee} \) are located in the Section 4.9.4. The integrated fluorescence areas were all determined by numerical integration of fluorescence area using A|E UV-Vis-IR Spectral Software which is further discussed in the Section 4.9.5.\(^{29} \)

4.4.3 Antenna Effect

The devices antenna effect (\( AE \)) is a measure of the devices light collecting ability.\(^{30,31} \) The \( AE \) 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.\(^{32} \) For our
investigation the AE is the ratio between the fluorescence intensity emitted from the terminal Cy5.5 acceptors when the devices are excited with 515 nm and 685 nm wavelength light. When the device is excited with 515 nm light the Cy3s fluorophores are directly excited. They in turn transfer their energy in a stepwise manner to the terminal Cy5.5 fluorophores. Thus, the devices act as light collecting antennas. In other words, the AE is a metric to evaluate how the device’s light collecting efficiency increases as more donors are added, providing a measure of the efficiency within the same device. Our investigation found that the AE could be found using two methods, one by exciting the devices with 515 nm and then 685 nm light (515/685 nm) and the other by exciting the devices with 515 nm light and then 635 nm light, (515/635 nm). When the second method was used, the AE was found to be half that of the 515/685 nm AE.

The second method, which we defined as the experimental antenna effect (AE_E), had the advantage that the entire Cy5.5 peak could be collected. This was not possible when exciting the devices with 685 nm light due to the spectrometers bandwidth cutoffs. The AE_E is defined in Equation (4.19). Where \( I_{Cy5.5, 515 \, nm} \) and \( I_{Cy5.5, 635 \, nm} \) are the fluorescence intensities of the terminal Cy5.5 following the direct excitation of the initial Cy3 donor at 515 nm and at 635 nm.

\[
AE_E = \frac{I_{Cy5.5, 515 \, nm}}{I_{Cy5.5, 635 \, nm}}
\]

(4.19)

The \( AE_E \) is the ratio between the fluorescence intensity of the terminal Cy5.5 acceptor excited at 515 nm in the presence of all the dyes to the direct excitation of the Cy5.5 acceptor at 635 nm. In addition to determining the \( AE_E \), the 700 nm fluorescence
excitation spectra was collected for each device throughout the wavelength range of 400-690 nm. The excitation spectra was integrated and the area was designated as the $E_W$. The $E_W$ represents the total amount of light the devices are capable of collecting independent of the wavelength. The $E_W$ parameter is closer to how a dye-doped solar cell functions if all the white light entering the system was absorbed.\textsuperscript{33}

### 4.4.4 Simplified HomoFRET Model

To determine how the devices overall absorbance and efficiency change as the homoFRET region was extended we devised the following model. We began focusing on the changes in efficiency as Cy3 dyes are added to the homoFRET region. Ignoring any increase in the overall absorbance the efficiency is given by Equation (4.20). Where $\lambda_I$, and $\lambda_F$ are the initial and final wavelengths, respectively, $d$ represents the dye, $i = (\text{AF}488, \text{Cy}3, \text{Cy}3.5, \text{Cy}5, \text{Cy}5.5)$, and $R$ is the number of Cy3 repeats.

\[
\text{Efficiency} = \int_{\lambda_I}^{\lambda_F} \sum_d P(d_i, R) \ast E_{ee}(d_i, R) \ d\lambda \tag{4.20}
\]

For $AE$ we set $\lambda_I$, and $\lambda_F$ at 513 and 517 nm, respectively, to account for the 5 nm slit width. For the $E_W$ we set $\lambda_I$, and $\lambda_F$ at 400 to 690 nm, respectively. Equation (4.21) gives the probability of exciting a chosen dye as a function of the $R$.

Where $[d_i]$ is the relative concentration, and $\epsilon_{d_i}^\lambda$ is the extinction coefficient of the dye at the specific wavelength. To account of the number of arms in each structures, $[d_{Cy3}]$ takes the value of $R \ast k$, where $k$ is 8 for the eight-arm star, and dendrimer, and 4 for the four-arm star.

\[
P(d_i, R) = \frac{[d_i] \ast \epsilon_{d_i}^\lambda}{\sum [d_i] \ast \epsilon_{d_i}} \tag{4.21}
\]
Here we assume one is using the relative concentrations and does not multiply by the device concentration as well. The probability density function in this case is 1 if we assume that this deals with absorbed photons and every absorbed photon was absorbed by one of the five dyes. The estimated end-to-end efficiency \( (E_{ee}) \) of an exciton starting on a particular dye for each of the structures is given by their respective Equations (4.22)-(4.25). Where \( E_{ee}(AF488, R) \), \( E_{Cy3\rightarrow Cy3.5}(R) \), \( E_{ee}(Cy3, R) \), \( E_{Cy3.5\rightarrow Cy5} \), and \( E_{Cy5\rightarrow Cy5.5} \) represent the respective experimentally determined FRET step efficiency values that are listed in the Section 4.9.7.

\[
E_{ee}(AF488, R) = \left[ E_{ee}(AF488, R) \times E_{Cy3\rightarrow Cy3.5}(R) \right] \tag{4.22}
\]
\[
E_{ee}(Cy3, R) = \left[ E_{ee}(Cy3, R) \right] \tag{4.23}
\]
\[
E_{ee}(Cy3.5) = \left[ E_{Cy3.5\rightarrow Cy5} \times E_{Cy5\rightarrow Cy5.5} \right] \tag{4.24}
\]
\[
E_{ee}(Cy5) = \left[ E_{Cy5\rightarrow Cy5.5} \right] \tag{4.25}
\]

We assume the only efficiency modified by \( R \) is \( E_{Cy3\rightarrow Cy3.5} \). Therefore, only \( E_{ee}(AF488, R) \) and \( E_{ee}(Cy3, R) \) are modified by \( R \). We keep in mind that \( R \) will be affected in regions where the dyes have absorbance. Generally speaking, the overall \( AE \) will decrease as \( R \) is increased independently of whether a small excitation window, in the case of the \( AE \), or the large excitation window, in the case of \( E_W \), is used. As \( R \) is increased the \( E_{ee} \) will also decrease with each additional step. We initially assumed the low \( E_{ee} \) would be counterbalanced by the increase in overall photons that were absorbed. Therefore, we assume that the number of photons absorbed is given by Equation (4.26).
Absorption = \int_{\lambda_l}^{\lambda_F} \sum_{d_i} [d_i] \ast \epsilon_{d_i}^\lambda \ d\lambda \quad (4.26)

The AE is given by multiplying the efficiency in Equation (4.20) by the number of photons absorbed in Equation (4.26).

\[ AE = \int_{\lambda_l}^{\lambda_F} P(d_i, R) \ast E_{ee}(d_i, R) \ast \sum_{d_i} [d_i] \ast \epsilon_{d_i}^\lambda \ d\lambda \quad (4.27) \]

4.4.5 Monte Carlo Simulations

To better understand the energy transfer, detailed Monte Carlo simulations were carried out. The simulations assumed that the fluorophores interacted solely via dipole-dipole coupling. The DNA was modeled as a fixed ring with straight arms that swung freely at their attachment points. The dye positions are based on the DNA design with linkers whose angles vary only in azimuth around the DNA and the dipoles are assumed static. The formation yield of each dye was used as a fitting parameter to match the four-arm star, eight-arm star, and dendrimer pair. The model has been previously described so only a brief description will be presented here.\footnote{4,15}

Since we treated target structures as incompletely formed and accompanied by assorted partial constructs/free dyes, we normalized the governing rate equations by the total concentration and the variables then become equivalent to the probabilities. For steady-state, only the time-integrated probability \( W_{ik} \) that the \( i \)th dye on the \( k_{th} \) construct will be excited is needed, and one can show that this obeys Equation (4.28).

\[
W_{ik} = \frac{1}{S} \sum_{m} \Delta_{ik}^m \sum_{d_i} P(d_i, R) \ast E_{ee}(d_i, R) \ast [d_i] \ast \epsilon_{d_i}^\lambda \, d\lambda
\]

Where \( S \) is the number of different constructs in the ensemble, \( M \) is the number of different types of dyes, \( N_k \) is the number of dyes in the \( k \)th construct, \( \Delta_{ik}^m \) is unity only if dye \( i \) on construct \( k \) is of type \( m \).
\[ W_{ik} \left[ 1 + \sum_{j=i}^{N_k} b_{ij}^k \right] - \sum_{j=1}^{N_k} b_{ij}^k W_{jk} = \sum_{m=1}^{M} \Delta_{ik}^m \eta_m, \ i = 1, ..., N_k, k = 1, ..., S \] (4.28)

The matrix element \( b_{ij}^k \) specifies the excitonic coupling between dyes \( i \) and \( j \) on construct \( k \), and according to Förster theory it varies as \( 1/r^6 \) (\( r \)=inter-dye distance).

The quantities in Equation (4.28) are related to the fluorescent intensity areas \( \Phi_m \) by Equation (4.29). Where \( Q_m \) is the quantum yield of dye \( m \), \( \rho(k) \) is the molar concentration of construct \( k \), and \( N_{mk} \) is the number of dyes of type \( m \) in construct \( k \).

\[
\Phi_m = \Psi Q_m \left[ 1 + \sum_{k=1}^{S} \rho(k) \sum_{i=1}^{N_k} \Delta_{ij}^m W_{jk} + \left( N_{m,\text{ideal}}^{(1)} - \sum_{k=1}^{S} \rho(k) N_{k}^{m,\text{ideal}} \right) \eta_m \right] m = 1, ..., M
\] (4.29)

4.5 Results

The photophysical properties of the fluorophores were calculated using the collected absorbance and fluorescence spectra for each fluorophore as described in Section 4.4. Table 4.1 lists the experimentally determined quantum yields (QY), the extinction coefficients (\( \epsilon \)), the wavelengths for the fluorophores peak absorbance \( (\text{Abs}_{\text{max}}) \) and emission \( (\text{Em}_{\text{max}}) \), the overlap integral \( (J) \), and the Förster radius, \( (R_0) \) for each fluorophore. The QY determination were described in Section 4.3.1.

<table>
<thead>
<tr>
<th>AF488</th>
<th>Cy 3</th>
<th>Cy 3.5</th>
<th>Cy 5</th>
<th>Cy 5.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.82</td>
<td>71,000</td>
<td>495</td>
<td>519</td>
<td>9.2 \times 10^{14}/4.6</td>
</tr>
<tr>
<td>0.19</td>
<td>150,000</td>
<td>550</td>
<td>570</td>
<td>-</td>
</tr>
<tr>
<td>0.17</td>
<td>150,000</td>
<td>581</td>
<td>596</td>
<td>-</td>
</tr>
<tr>
<td>0.28</td>
<td>250,000</td>
<td>649</td>
<td>670</td>
<td>-</td>
</tr>
<tr>
<td>0.55</td>
<td>190,000</td>
<td>675</td>
<td>694</td>
<td>-</td>
</tr>
</tbody>
</table>

\( ^a \) The average of experimentally determined QY for each DNA labeled fluorophore.

\( ^b \) Förster distances \( (R_0) \) were calculated assuming a 2/3 value for \( \kappa \) and 1.33 value for \( n \).
4.5.1 Device Formation Yield

The formation yield of the devices was evaluated by gel electrophoresis. Each device and its respective R1 through R6 counterpart were evaluated. The gels are shown in Figure 4.3. The gels were stained using Gel Red stain as a fluorescent marker and a way of determining the relative amount of DNA present in each gel band. The formation was also estimated by integrating the area of each peak and then dividing the area of the peak containing the device by the total area of the bands in the specific gel lane. The four-arm star device was shown to have the highest formation yield of approximately 90% for the R1 through R3 devices and approximately 85% for the R4 through R6 eight-arm arm devices. The dendrimer device formation efficiency followed the same gradually decreasing trend as the four-arm device but was also found to have an approximately 20% lower overall formation efficiency for devices R1 through R6. This apparent trend is shown in Figure 4.3d. The eight-arm device formation efficiency ranged from 45% for the R1 device to approximately 20% for the R6 device. In general, as the device’s linear dimension increased, the formation efficiency decreased.
Figure 4.3. (a-c) 3.0 % Agarose gel electrophoresis of the three devices, four-arm star, eight-arm arm star, and dendrimer, respectively, to evaluate the formation yield. (d) A plot of the estimated device formation yield determined by analyzing the intensity of the gel images above. The four-arm star and the dendrimer device formed in relatively high yield. The eight-arm star was found to have the lowest formation yield.

4.5.2 Fluorescence Lifetime Measurements

The fluorescence decay of the devices was investigated using a time-correlated single photon counting (TCSPC) technique described in Section 4.3.5. The devices exhibit a distribution of fluorescence decay rates due to the noncovalent ensemble effects. An average of the fluorescence decay rates can be used to give an estimate of
the devices performance. The fluorescence lifetimes of all the devices were observed to decrease as the length of the Cy3 homoFRET region was increased from one repeat (R1) to six repeats (R6). The four-arm star R1 and R6 decay rates were calculated to be $1.37 \pm 0.09$ ns and $1.04 \pm 0.02$ ns, respectively. Whereas the decay rates for the R1 and R6 eight-arm star were $1.32 \pm 0.04$ ns and $1.09 \pm 0.02$ ns, and the dendrimer values were $1.59 \pm 0.12$ ns and $1.19 \pm 0.04$ ns. We also observed a rapid decrease in lifetime from R1 to R2 that correspond to a much slower rate afterwards.

4.5.3 Fluorescence Lifetime Anisotropy Measurements

Anisotropy measurements were collected to confirm the presence of homoFRET transfer in the devices. Anisotropy measurements were used to determine the fluorescence lifetime of the Cy3 fluorophores. As expected the anisotropies decrease from R1 to R6. The decrease can be observed in Figure 4.4 and the calculated fluorescence lifetime anisotropies are shown in Table 4.2. The anisotropy was used to determine the transfer efficiency for the homoFRET Cy3-Cy3 region. The homoFRET step efficiency was found to be $0.89 \pm 0.18$, $0.76 \pm 0.19$, and $0.83 \pm 0.19$, for the four-arm star, eight-arm star, and dendrimer, respectively. As the Cy3 homoFRET region is lengthened from R1 to R6, the anisotropy is expected to decrease as a result of the misalignment of individual dye dipoles between the donors and acceptors. The fluorescence lifetime anisotropy decay rates for the full device vs. single arm devices were also measured to determine the effect of additional arms on the devices light-harvesting capabilities as described in Section 4.9.3.
Table 4.2. The experimentally determined fluorescence lifetime anisotropy for all the devices.

<table>
<thead>
<tr>
<th>Device</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Arm Star</td>
<td>4.03 ± 0.43</td>
<td>1.02 ± 0.11</td>
<td>0.73 ± 0.07</td>
<td>0.82 ± 0.06</td>
<td>0.72 ± 0.07</td>
<td>0.65 ± 0.08</td>
</tr>
<tr>
<td>8-Arm Star</td>
<td>4.26 ± 0.41</td>
<td>1.07 ± 0.10</td>
<td>0.71 ± 0.07</td>
<td>0.62 ± 0.08</td>
<td>0.69 ± 0.07</td>
<td>0.75 ± 0.07</td>
</tr>
<tr>
<td>Dendrimer</td>
<td>4.00 ± 0.40</td>
<td>1.09 ± 0.11</td>
<td>0.87 ± 0.09</td>
<td>0.53 ± 0.05</td>
<td>0.50 ± 0.05</td>
<td>0.50 ± 0.05</td>
</tr>
</tbody>
</table>

The fluorescence lifetime anisotropy decay rates for the full device vs. single arm devices and found that the full devices had faster decay rates. The additional arm provides additional homoFRET and therefore likely hetreoFRET pathways between arms. The fluorescence anisotropy for R1 stars was found to be slightly higher than the same values for R2 in the free-floating arms. The lifetime anisotropy investigations for the free-floating arms can be found in Section 4.9.8.
Figure 4.4.  Fluorescence lifetime anisotropy spectra for the Cy3 in the AF488-Cy5.5 devices.  (a-c) The raw fluorescent lifetimes for the four-arm star, eight-arm star, and dendrimer devices.  As expected the decay rate rapidly increased from R1 to R2 and then slowly approach the R6 decay rate.  (d) A plot of the determined anisotropies lifetimes in units of ns.

4.5.4 Steady-state Fluorescence Spectra

Averaged steady-state fluorescence spectra from the three devices are shown in Figure 4.5.  Averaging the individual spectra from the respective devices collected during three independent experiments created the plots.  Figures 4.5a-c are averaged spectra from representative four-arm star, eight-arm star, and dendrimer devices with extensions R1 through R6, respectively.  Figures 4.5d-f depict only the R6 device from
each respective data set along with the deconvoluted spectral components. The individual Cy5.5 sensitized emission components are shown in blue along with the raw spectra that are shown in black for each respective device. The integrated area of the Cy5.5 peak represents the number of photons that were transferred from the AF488 donors. The complete set the averaged steady-state fluorescence spectra can be found in Sections 4.9.13 through 4.9.21.

![Figure 4.5](image)

Figure 4.5. (a-c) The averaged fluorescent spectra for the four-arm star, eight-arm star, and dendrimer devices, R1-R6, respectively. (d-f) The deconvoluted spectra for the R6 device of each respective device are shown to the right. The Cy5.5 sensitized emission for each respective device is shown in the inset.

Throughout the investigation, we analyzed 180 unique device configurations. Each device configuration was synthesized and characterized a minimum of three
Each experimental run was independent, meaning the devices were synthesized and characterized independently of each other and the experiments were conducted at different times. The process of preparing and characterizing the different device configurations was expedited as a result of the devices modular DNA design. Additionally, the Tecan plate reader allowed for automated spectra collection of up to 96 samples.

4.5.5 Transfer Analysis

4.5.5.1 End-to-End Efficiency

As seen in photonic wires the $E_{ee}$ decreased as the homoFRET region was extended from one to six Cy3 repeats. The $E_{ee}$ for the R1 through R6 are plotted in Figure 4.6. In the case of the dendrimer, the $E_{ee}$ ranged from 16.5 ± 2.4% to 1.8 ± 1.1% for the R1-R6 devices. The four-arm star ranged from 8.9 ± 1.4% to 3.3 ± 1.2% and the eight-arm star had the lowest efficiency and ranged from 6.9 ± 0.5% to 0.4 ± 0.3%.

![End-to-End Transfer Efficiency](image)

Figure 4.6. A plot of the $E_{ee}$ for 488-Cy5.5 devices R1 through R6. The dendrimer was shown to have the highest efficiency followed by the four-arm star and finally the eight-arm star device. The black error bars represent the absolute experimental error in each measurement.
Table 4.3. Experimentally determined end-to-end efficiencies for all the devices.

<table>
<thead>
<tr>
<th>Device</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (nm)</td>
<td>12.0</td>
<td>15.1</td>
<td>18.2</td>
<td>21.3</td>
<td>24.4</td>
<td>27.5</td>
</tr>
<tr>
<td>4 Arm Star</td>
<td>8.9 ± 1.4</td>
<td>5.4 ± 1.5</td>
<td>4.6 ± 0.5</td>
<td>3.9 ± 1.1</td>
<td>3.0 ± 0.5</td>
<td>3.3 ± 1.2</td>
</tr>
<tr>
<td>8 Arm Star</td>
<td>6.9 ± 0.5</td>
<td>4.8 ± 0.5</td>
<td>3.1 ± 0.4</td>
<td>2.4 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>Dendrimer</td>
<td>16.5 ± 2.4</td>
<td>9.4 ± 1.3</td>
<td>7.0 ± 1.5</td>
<td>4.2 ± 1.7</td>
<td>2.7 ± 1.1</td>
<td>1.8 ± 1.1</td>
</tr>
</tbody>
</table>

*a Estimated length based on number of base pairs.

Plots comparing $E_{ee}$ to $E_{ae}$ are shown in Section 4.9.4. In general, large deviations occurred between $E_{ee}$ and $E_{ae}$. The deviations were attributed to the fact that $E_{ae}$ did not correct for energy which was transferred as a result of the Cy3 emission to the Cy3.5, Cy5, and Cy5.5. The difference between $E_{ee}$ and $E_{ae}$ was observed to increase as the homoFRET regions were increased from one to six. The increase results from the increased indirect excitation of the Cy3 which is transferred to the terminal Cy5.5.

4.5.5.2 Antenna Effect

![Figure 4.7](image)

Figure 4.7. (a) The experimental antenna effect $AE_E$ for each device. The $AE_E$ is the increase or decrease in the acceptor emission resulting from a change in the donor. (b) The normalized integrated emission spectra collected at 700 nm from the devices, $E_W$. 
The $AE$ was experimentally found to be exactly double of the $AE_E$ ($2.0 \pm 0.1$). See Section 4.9.9 for a complete comparison. The $AE$ for the four-arm and eight-arm star devices is almost the same however the eight-arm star device is slightly higher as can be seen in Figure 4.7. The $E_W$ for the dendrimer and the eight-arm star outperformed the four-arm star device.

4.5.5.3 Antenna Effect Model

The project was undertaken with the expectation that homoFRET regions would increase the energy harvesting capabilities of the structures up to a point. The $AE$ calculation was used as a simple way of estimating how often the devices were excited, what dyes were excited, and how efficient the energy transfer was to the final dye. The results from the $AE$ calculation are shown in Figure 4.8. Plots of the calculated $AE$ and $E_W$ are shown in Figures 4.8a and 4.8b, respectively.

The calculated $AE$ for the dendrimer device was found to rapidly increases from R1 to R3 where, at R3, it reaches a maximum compared to the four-arm star which slowly increases from R1 to R4 and reaches a maximum around R4. The eight-arm star generally linearly decreases from R1 to R6. The $E_W$ follows a similar trend, however, the peak values are shifted to higher repeat numbers with the dendrimer reaching a maximum $AE$ for the R4 device. The model is compared to the experimentally collected values for the stars and the dendrimer devices in Figures 4.8c and 4.8d. The model generally predicts the total transfer efficiency for the four-arm and eight-arm stars however the model begins to break down after the R3 devices. The model was found not to correctly predict the dendrimers transfer efficiencies, especially for the R2 through R5 devices.
Figure 4.8. (a-b) Plots of the calculated $AE$ and $E_W$ for all the devices, respectively. (c) The calculated transfer efficiency for the four-arm and eight-arm star devices. (d) The calculated transfer efficiency for dendrimer devices. The experimentally determined and calculated values for the transfer efficiency are depicted as squares and circles, respectively. The model generally predicts the transfer efficiency for the star devices, however, it fails to correctly predict the dendrimers transfer efficiencies, especially for the R2 through R5 devices.

4.5.6 Monte Carlo Simulations

Monte Carlo simulations were used to better understand the energy transfer in the devices. The model was described in Section 4.4.5. The dendrimer simulations
are shown in Figure 4.11. Simulations for the four-arm and eight-arm stars are shown in Figures 4.9 and 4.10, respectively. The Monte Carlo simulations were fit to the individual FRET step efficiencies for each dye pair in each device. Using the set step efficiencies, the R1 through R6 devices were then modeled. The simulations for the four-arm star were fit assuming the following dye yields: Cy3 ~ 85%, Cy3.5 ~ 63%, Cy5 ~ 15% and Cy5.5 ~ 65%. For the model to represent the experiment, the formation yield of Cy5 was assumed to be particularly poor. The simulations for the eight-arm star were fit assuming the following dye yields: Cy3 ~ 53%, Cy3.5 ~ 67%, Cy5 ~ 12% and Cy5.5 ~ 43%.

**FRET Monte Carlo Simulations Four-Arm Star**

![Diagram](image)

**Figure 4.9.** Monte Carlo FRET simulations for the four-arm star device. (a) A plot of the simulated dye pair FRET efficiency for each heteroFRET step. The dashed line and solid line represent an ideally formed device and a non-ideally formed device, respectively. (b) A plot of the simulated end-to-end efficiencies for the four-arm star R1 through R6 devices. The simulation used the dye pair transfer efficiencies shown in (a). In both plots, the red and blue squares represent the measurement.
Figure 4.10. Monte Carlo FRET simulations for the eight-arm star device. (a) A plot of the simulated FRET step efficiency for each heteroFRET step. (b) A plot of the simulated end-to-end efficiencies for the eight-arm star R1 through R6 devices. The simulation used the dye pair transfer efficiencies shown in (a). In both plots, the red and blue squares represent the measurement.

The model for the eight-arm star does predict the transfer efficiency for the R3 through R6 devices and is slightly better than the modeling for the four-arm star devices. The Monte Carlo simulations for the dendrimer device is shown in Figure 4.11. The model was found to fit the experimentally collected transfer efficiency when a 75% formation efficiency was assumed. Meaning that 25% of the fluorophores were detached from the device and free floating in the solution.
Figure 4.11. Monte Carlo FRET simulations for the dendrimer device. The red and blue dashed lines represent the model assuming 100% and 75% device formation yields, respectively. The green squares are the experimentally determined transfer efficiency. The model was fit assuming a device formation yield of 75%.

4.6 Discussion

For all devices, the antenna effect was observed to increase as the number of arms or branches was increased from four to eight. The additional Cy3 fluorophores enabled higher light-collection efficiency. In general, the investigation showed that five Cy3 repeats in the homoFRET region resulted in the highest antenna effect despite the dendrimer R6 device’s antenna gain be slightly higher than that of the R5 device. The devices’ high gain was attributed to the cores’ more efficient redundant design and the increased dye branching ratio. The design is redundant in the sense that the device would still function even if some fluorophores were detached from the DNA template. The four-arm and eight-arm stars do not share this ability. For
example, if one fluorophore is missing the homoFRET energy propagation path is effectively cut off. The Monte Carlo modeling revealed that around 25% of the devices’ fluorophores are likely detached from the DNA template; thus, the error-correcting designs are advantageous. Post device formation purification could be employed to help reduce the number of free-floating dyes. However, the additional purification step reduces the overall device concentration and could damage the devices.

When comparing the four-arm and eight-arm stars, the devices’ end-to-end and formation efficiencies should be considered. The relatively poor formation yields of the eight-arm star devices likely resulted from the relatively high conformational stress associated with the formation of the eight-arm junction. Previous studies have revealed that continuous 16 bp hybridization regions are required along each arm to achieve high DNA template formation yield. However, using long continuous strands would not allow for dye arrangements with the spaces required for efficient FRET. Previous investigations have shown that the eight-arm star is generated with a characterizable formation yield when each arm is formed with discontinuous binding regions. In contrast to the eight-arm stars’ formation efficiency, the four-arm star device exhibited the highest efficiency among the devices investigated. The four-arm junction’s high formation efficiency was attributed to the junction’s ability to form without high DNA bending stresses. The dendrimer’s formation efficiency was found to be between that of the four-arm and eight-arm star devices.

In general, the results revealed that homoFRET led to a slight increase in efficiency, as was observed in previous investigations. However, competing
problems with non-directionality, DNA quenching, device formation yield, and the lack of excitation of all structures during the steady-state fluorescence measurements are thought to have been mainly responsible for the relatively lower-than-expected performance observed. The non-directional nature of homoFRET cannot be overcome, so we will focus on the other factors. The second issue relates to the fluorescence measurement technique. Calculations have revealed that the fluorimeters’ excitation intensity is relatively low; thus, only a small fraction of the devices can be excited. In fact, estimates suggest that only 1 in 20 devices are excited. One solution would be to excite the devices with a laser; however, spectral broadening could occur as laser intensity increases, which is problematic. The anisotropy measurements clearly showed that homoFRET pathways function to transfer energy in the devices, which was evident in the decrease in anisotropy as the homoFRET region was extended. Anisotropy can primarily be decreased by two mechanisms, namely restricting the samples’ rotational diffusion and the presence of homoFRET. The anisotropy was observed to decrease as the homoFRET region was extended from R1 to R6 for all of the devices. This observation is consistent with previous work where a decrease in anisotropy was observed as a homoFRET region comprising Cy3.5 fluorophores was lengthened. The anisotropy measurements also revealed that the Cy3-Cy3 homoFRET step efficiency, which was calculated from the devices’ anisotropy decay, was relatively constant at around 80% efficiency.

The antenna effect model predicted an increase in the $AE$ and $E_W$ as additional dyes were added for the four-arm star and dendrimer device as was
shown in Figure 4.8. The model and experimental data both show initial increases in $AE$ and $E_W$ with a maximum obtained at R4 for the dendrimers and R5 for the four-arm star, the eight-arm star predicts a maximum at only R2 due to the inefficiency of formation of the higher R structures which have a larger effect on the efficiency. The reason for the discrepancy in peak positions results from the fact that the $AE$ wavelength region (513-517 nm) is much more narrow that the $E_W$ wavelength region (400-690 nm). Because the $E_W$ has a broad wavelength region, it is not greatly modified by increasing Cy3 dyes as much as is the $AE$. Considering all these factors the $AE$ is much more susceptible to the extension of the homoFRET region than the $E_W$. The $AE$, for example, has a 50% increase from its minimum to the maximum value compared only a 10% increase for the $E_W$. These results are mirrored by the model that shows a smaller amount of gain for the star devices compared to the dendrimer devices. This relatively simple model can, therefore, be used to predict optimal outputs by modifying the number of repeats depending on the chosen excitation wavelengths.

4.7 Conclusion

In conclusion, we designed, constructed, and characterized three relatively complex FRET networks with configurable homoFRET sections. We demonstrated that DNA-directed self-assembly is a viable bottom-up manufacturing technique for the construction of light-harvesting devices. HomoFRET regions were shown to be able to increase the devices’ antenna gains. A remarkably high antenna gain of 5.7 was observed in the dendrimer device. This was primarily attributed to the presence
of a homoFRET region but also the 2:1 branching ratio in the dendrimer device. In
general, the highest antenna gains were observed with five Cy3 homoFRET dye
repeats. The work showcased DNA structural technologies’ powerful and versatile
ability to devise complex FRET networks, biosensors, and light-harvesting devices.

4.8 Acknowledgments

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recognize the NRL Nanosciences Institute and DTRA JSTO MIPR # B112582M.
4.9 Supporting Information

4.9.1 DNA Sequences and Design Schematics

Table 4.4. The DNA sequences for the dendrimer device.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>bps</th>
<th>Mp.1,2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Den Cap</td>
<td>AF488-ACGCGACTT-TACGTGTCG-AF488</td>
<td>18</td>
<td>56.3</td>
</tr>
<tr>
<td>Den Cap Un</td>
<td>ACGCGACTT-TACGTGTCG</td>
<td>18</td>
<td>56.3</td>
</tr>
<tr>
<td>R1 AF488</td>
<td>AF488-GATGCACAT-TCGTCCCT-AF488</td>
<td>18</td>
<td>53.8</td>
</tr>
<tr>
<td>R1 AF488 Un</td>
<td>GATGCACAT-TCGTCCCT</td>
<td>18</td>
<td>53.8</td>
</tr>
<tr>
<td>Cy3 Rep</td>
<td>CGACACGTA-Cy3</td>
<td>9</td>
<td>28.0</td>
</tr>
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<td>Cy3 Rep Un</td>
<td>CGACACGTA</td>
<td>9</td>
<td>28.0</td>
</tr>
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<td>2Cy3</td>
<td>AGGGAAACGA-Cy3-AGAAGAGAGACAGGGAG-Cy3-ATGTGCATC</td>
<td>33</td>
<td>75.4</td>
</tr>
<tr>
<td>2Cy3 Un</td>
<td>AGGGAAACGA-AGAAGAGACAGGGAGATGTGCATC</td>
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<td>75.4</td>
</tr>
<tr>
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<td>85.3</td>
</tr>
<tr>
<td>Cy3.5 Un</td>
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<td>58</td>
<td>85.3</td>
</tr>
<tr>
<td>Cy5</td>
<td>AGGGAAACGAACTCCCTGTATCCCGTGACT-Cy5-TAATCGTGAG</td>
<td>76</td>
<td>89.0</td>
</tr>
<tr>
<td>Cy5 Un</td>
<td>AGGGAAACGA-AGAAGAGACAGGGGATTCTCTCATATGTGCATC</td>
<td>77</td>
<td>89.0</td>
</tr>
<tr>
<td>2Cy5.5</td>
<td>AGGGAAACGAACTCCCTGTATCCCGTGACT-Cy5-ATCAG-GGTGATCTCTCTCTCTCTCTATGTGCATC</td>
<td>76</td>
<td>89.0</td>
</tr>
<tr>
<td>2Cy5.5 Un</td>
<td>AGGGAAACGA-AGAAGAGACAGGGGATTCTCTCATATGTGCATC</td>
<td>77</td>
<td>89.0</td>
</tr>
</tbody>
</table>

1 Calculated melting point (Mp) of ssDNA strands. Oligonucleotide Properties Calculator.
2 The salt adjusted melting temperature was used for all Mp calculations.
### Table 4.5. The DNA sequences for the extendable homoFRET region for the dendrimer device.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>bps</th>
<th>Mp.(^1,2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 AF488</td>
<td>AF488-GATGCACAT-TCGTTCCT- AF488</td>
<td>18</td>
<td>53.8</td>
</tr>
<tr>
<td>R1 AF488 Un</td>
<td>GATGCACAT-TCGTTCCT</td>
<td>18</td>
<td>53.8</td>
</tr>
<tr>
<td>DenTemp R2</td>
<td>GATGCACATTCGTTCCT-AAGTCCCGT</td>
<td>27</td>
<td>69.9</td>
</tr>
<tr>
<td>DenTemp R3</td>
<td>GATGCACATTCGTTCCT-TACGTGTCG-AAGTCCCGT</td>
<td>36</td>
<td>77.9</td>
</tr>
<tr>
<td>DenTemp R4</td>
<td>GATGCACATTCGTTCCT-TACGTGTCG-TACGTGTCG-AAGTCCCGT</td>
<td>45</td>
<td>82.4</td>
</tr>
<tr>
<td>DenTemp R5</td>
<td>GATGCACATTCGTTCCT-TACGTGTCG-TACGTGTCG-TACGTGTCG-AAGTCCCGT</td>
<td>54</td>
<td>85.9</td>
</tr>
<tr>
<td>DenTemp R6</td>
<td>GATGCACATTCGTTCCT-TACGTGTCG-TACGTGTCG-TACGTGTCG-AAGTCCCGT</td>
<td>63</td>
<td>88.0</td>
</tr>
</tbody>
</table>

\(^1\) Calculated melting point (Mp) of ssDNA strands. Oligonucleotide Properties Calculator.\(^2\)

\(^2\) The salt adjusted melting temperature was used for all Mp calculations.

#### 4.9.1.1 Four-Arm Star Device DNA Sequences

### Table 4.6. The DNA sequences for the four-arm star device.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>bps</th>
<th>Mp.(^1,2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Star Cap</td>
<td>ACGCGACTT-AAF488</td>
<td>9</td>
<td>28.0</td>
</tr>
<tr>
<td>Star Cap Un</td>
<td>ACGCGACTT</td>
<td>9</td>
<td>28.0</td>
</tr>
<tr>
<td>Cy3 Rep</td>
<td>CGACACGTA-Cy3</td>
<td>9</td>
<td>28.0</td>
</tr>
<tr>
<td>Cy3 Rep Un</td>
<td>CGACACGTA</td>
<td>9</td>
<td>28.0</td>
</tr>
<tr>
<td>B Cy3.5</td>
<td>AGCAGACTAT-Cy3-5-TGTCGCG</td>
<td>17</td>
<td>52.4</td>
</tr>
<tr>
<td>B Cy3.5 Un</td>
<td>AGCAGACTATATTGTGCG</td>
<td>17</td>
<td>52.4</td>
</tr>
<tr>
<td>L1</td>
<td>GG-Cy5-CCAGCGGTA-Cy5-CAGCGACGA</td>
<td>21</td>
<td>69.0</td>
</tr>
<tr>
<td>L1 Un</td>
<td>GGCACGAGGTGCCAGCAGA</td>
<td>21</td>
<td>69.0</td>
</tr>
<tr>
<td>L2</td>
<td>CCGATTGCG-Cy5.5-TGTCGACCGCGACGA</td>
<td>23</td>
<td>69.9</td>
</tr>
<tr>
<td>L2 un</td>
<td>CCGATTGCGTCGACCGCGACGA</td>
<td>23</td>
<td>69.9</td>
</tr>
<tr>
<td>L3</td>
<td>CG-Cy5-CCAGCGCAACAGGCAGA</td>
<td>21</td>
<td>71.0</td>
</tr>
<tr>
<td>L3 Un</td>
<td>GC-Cy5-CCAGCGCAACAGGCAGA</td>
<td>21</td>
<td>71.0</td>
</tr>
<tr>
<td>L4</td>
<td>TGGACACCGG-Cy5.5-TGTCGACCGCGACGA</td>
<td>22</td>
<td>70.0</td>
</tr>
<tr>
<td>L4 Un</td>
<td>TGGACACCGGTCGACCGCGACGA</td>
<td>23</td>
<td>71.9</td>
</tr>
</tbody>
</table>

\(^1\) Calculated melting point (Mp) of ssDNA strands. Oligonucleotide Properties Calculator.\(^2\)

\(^2\) The salt adjusted melting temperature was used for all Mp calculations.
### 4.9.1.2 Eight-Arm Star Device DNA Sequences

#### Table 4.7. The DNA sequences for the eight-arm star device.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>bps</th>
<th>Mp.¹,²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Star Cap</td>
<td>ACGCGACTT-AF488</td>
<td>9</td>
<td>28.0</td>
</tr>
<tr>
<td>Star Cap Un</td>
<td>ACGCGACTT</td>
<td>9</td>
<td>28.0</td>
</tr>
<tr>
<td>Cy3 Rep</td>
<td>CGACACGTA-Cy3</td>
<td>9</td>
<td>28.0</td>
</tr>
<tr>
<td>Cy3 Rep Un</td>
<td>CGACACGTA</td>
<td>9</td>
<td>28.0</td>
</tr>
<tr>
<td>B Cy3.5</td>
<td>AGCAGACTAT-Cy3.5-TCGTCGC</td>
<td>17</td>
<td>52.4</td>
</tr>
<tr>
<td>B Cy3.5 Un</td>
<td>AGCAGACTATTCGTCGC</td>
<td>17</td>
<td>52.4</td>
</tr>
<tr>
<td>L5</td>
<td>CGCAATCCT-Cy5.5-GGCGAGCGCGACGA</td>
<td>23</td>
<td>71.9</td>
</tr>
<tr>
<td>L5 Un</td>
<td>CGCAATCCTGGCGAGCGCGACGA</td>
<td>23</td>
<td>71.9</td>
</tr>
<tr>
<td>L6</td>
<td>GC-Cy5-CGCCATGAG-Cy5-GCGGCGACGA</td>
<td>21</td>
<td>71.0</td>
</tr>
<tr>
<td>L6 Un</td>
<td>GCCGCCATGAGCGCGACGA</td>
<td>21</td>
<td>71.0</td>
</tr>
<tr>
<td>L7 Un</td>
<td>CGCACTCAGCAGAAAGCGCGACGA</td>
<td>23</td>
<td>69.9</td>
</tr>
<tr>
<td>L8</td>
<td>GC-Cy5-TTCGCCCACTCy5-AGCGCGACGA</td>
<td>21</td>
<td>69.0</td>
</tr>
<tr>
<td>L8 Un</td>
<td>GCTTCGCCCACTCGCGACGA</td>
<td>21</td>
<td>69.0</td>
</tr>
<tr>
<td>L9</td>
<td>GCTAGTGGACACGACGACGACGA</td>
<td>23</td>
<td>69.9</td>
</tr>
<tr>
<td>L9 Un</td>
<td>GCTAGTGGACACGACGACGACGA</td>
<td>23</td>
<td>69.9</td>
</tr>
<tr>
<td>L10</td>
<td>GCTGCGGTCTGTGCGCGACGA</td>
<td>23</td>
<td>69.9</td>
</tr>
<tr>
<td>L10 Un</td>
<td>GCTGCGGTCTGTGCGCGACGA</td>
<td>23</td>
<td>69.9</td>
</tr>
<tr>
<td>L11 Un</td>
<td>GCCACAGTCTCAACGCGCGACGA</td>
<td>23</td>
<td>69.9</td>
</tr>
<tr>
<td>L12</td>
<td>CG-Cy5-TGAGGGGAT-Cy5-GCGGCGACGA</td>
<td>21</td>
<td>69.0</td>
</tr>
<tr>
<td>L12 Un</td>
<td>CGTGAAGGGATGCGCGACGA</td>
<td>21</td>
<td>69.0</td>
</tr>
</tbody>
</table>

¹ Calculated melting point (Mp) of ssDNA strands. Oligonucleotide Properties Calculator.
² The salt adjusted melting temperature was used for all Mp calculations.
### Table 4.8. The DNA sequences for the extendable homoFRET region for the four and eight-arm star devices.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>bps</th>
<th>Mp.¹,²</th>
</tr>
</thead>
<tbody>
<tr>
<td>StarTemp1</td>
<td>ATAGTCTGCT-TACGTGTCG-TACGTGTCG-AAGTCGCGT</td>
<td>29</td>
<td>70.1</td>
</tr>
<tr>
<td>StarTemp2</td>
<td>ATAGTCTGCT-TACGTGTCG-TACGTGTCG-TACGTGTCG-AAGTCGCGT</td>
<td>37</td>
<td>77.6</td>
</tr>
<tr>
<td>StarTemp3</td>
<td>ATAGTCTGCT-TACGTGTCG-TACGTGTCG-TACGTGTCG-TACGTGTCG-AAGTCGCGT</td>
<td>46</td>
<td>82.4</td>
</tr>
<tr>
<td>StarTemp4</td>
<td>ATAGTCTGCT-TACGTGTCG-TACGTGTCG-TACGTGTCG-TACGTGTCG-TACGTGTCG-TACGTGTCG-TACGTGTCG-AAGTCGCGT</td>
<td>55</td>
<td>85.7</td>
</tr>
<tr>
<td>StarTemp5</td>
<td>ATAGTCTGCT-TACGTGTCG-TACGTGTCG-TACGTGTCG-TACGTGTCG-TACGTGTCG-TACGTGTCG-TACGTGTCG-AAGTCGCGT</td>
<td>65</td>
<td>87.8</td>
</tr>
<tr>
<td>StarTemp6</td>
<td>ATAGTCTGCT-TACGTGTCG-TACGTGTCG-TACGTGTCG-TACGTGTCG-TACGTGTCG-TACGTGTCG-TACGTGTCG-AAGTCGCGT</td>
<td>74</td>
<td>89.4</td>
</tr>
</tbody>
</table>

¹ Calculated melting point (Mp) of ssDNA strands. Oligonucleotide Properties Calculator.² The salt adjusted melting temperature was used for all Mp calculations.
4.9.2  Design Schematics

4.9.2.1  Schematic of Four-arm Star Device

4 Arm Star R1

5’->3’
Star Cap  ACGCGACTT-AF488
Cys Rep  CGACACGT-Cy3
B Cys 5-AGCAGACTAT-Cys 5-TCGTCGC
L1  GG-Cys 5-CACGAGGTG-Cys 5-CGACGACGA
L2  CCGATTGC-Cys 5-CGTCGACCGACGA
L3  GC-Cys 5-CGACGCAGA-Cys 5-CGACGACGA
L4  TGGACACCG-Cys 5-TCGAGCAGCAGACGA
Star Temp 1  ATAGCTGCTT-TACGTCGC-AAGCAGT

T = Location of the T base replaced by the Cy5 un linker
Orange represents location of Cy5.5

Figure 4.12.  Schematic of the four-arm star device.
4.9.2.2 Schematic of Eight-arm Star Device

Figure 4.13. Schematic of the eight-arm star device.
4.9.2.3 Schematic of Dendrimer Device

a) Dendrimer R1

b) Dendrimer R2

Figure 4.14. Schematic of the R1 and R2 dendrimer devices.
4.9.3 Fluorescence Lifetime Measurements Equations

For the fluorescence lifetime determinations a biexponential decay function which accounts for two lifetimes and an intensity average, Equation (4.30) was used to fit the spectra. Where, \( a_1 \) and \( a_2 \) are fitting parameters and \( \tau_1 \) and \( \tau_2 \) are the lifetimes in units of ns.

\[
y(t) = a_1 \cdot e^{-t/\tau_1} + a_2 \cdot e^{-t/\tau_2}
\]  

Equation (4.30)

Equation (4.31), a double exponential decay equation was used to fit the fluorescence lifetime anisotropies spectra. The double exponential decay equation takes into account the movement of the molecule with the linker and the tumbling of the entire DNA device. Where, \( a_1 \) and \( a_2 \) are fitting parameters, \( \tau_1 \) and \( \tau_2 \) are interpreted as the rotational correlation times, and \( y_0 \) is the residual anisotropy

\[
y(t) = a_1 \cdot e^{-t/\tau_1} + a_2 \cdot e^{-t/\tau_2} + y_0
\]  

The average lifetime was found using Equation (4.32).\(^{20}\) Where, \( \alpha_1 \) and \( \alpha_2 \) are the preexponential terms which depend on the fluorophores concentration, absorption, and quantum yield.\(^{20}\)

\[
\tau_{\text{Avg}} = \frac{\alpha_1 \tau_1^2 + \alpha_2 \tau_2^2}{\alpha_1 \tau_1 + \alpha_2 \tau_2}
\]  

4.9.4 End-to-End Efficiency Comparison

In addition to calculating the \( E_{ee} \) we calculated a value for anywhere-to-end efficiency (\( E_{ae} \)) defined in Equation (4.17). The \( E_{ae} \) was calculated to show a
comparison between $E_{ee}$ and to give us an idea of the direct excitation that occurred throughout the devices in the Cy3 homoFRET region. Figure 4.15 is a plot comparing the two efficiencies for the three devices.

Figure 4.15. Plots comparing the anywhere-to-end, ($E_{ae}$) and the end-to-end efficiencies, ($E_{ee}$) for the three devices. (a-c) Plots of the efficiencies for the four-arm star, eight-arm star and dendrimer, respectively.

4.9.5 Spectral Decomposition

After the spectra from each device were collected each spectrum was imported into A|E UV-Vis-IR Spectral Software a freeware Matlab® program for
processing. The program allows the combined device spectra to be decomposed into its spectral components by providing the linear combination that best fits the composite spectra. An example is shown in Figure 4.16. The approach described above follows the analysis of FRET-based DNA photonic wires previously reported.\textsuperscript{4,36}

![Spectral Decomposition of Dendrimer R2 Construct](image)

**Figure 4.16.** A plot depicting the spectral decomposition process using A|E UV-Vis-IR Spectral Software. The collected spectrum is shown as a solid gray line whereas the fits are shown in dotted lines. The individual spectra components sum to create the collected spectra.

### 4.9.6 Absolute Error Calculation in $E_{\text{ee}}$ Efficiency

Propagation of standard uncertainties in combined functions involving division such as Equation (4.33),\textsuperscript{37}

$$F = \frac{x}{y} \quad (4.33)$$

yields the absolute uncertainty given by Equation (4.34).
\[ \sigma_F = \frac{x}{y} \sqrt{\left( \frac{\sigma_x}{x} \right)^2 + \left( \frac{\sigma_y}{y} \right)^2}. \] (4.34)

Equation (4.34) can be generalized to yield Equation (4.35) which gives the absolute uncertainty in the \( E_{\infty} \) FRET efficiency. Where \( \Phi_A \) and \( \Phi_D \) are the quantum yields (QY) of the terminal acceptor and donor, respectively. \( \phi_{AD} \) and \( \phi_D \) are the integrated fluorescence component area of the terminal acceptor in the presence of donor and donor only, respectively. \( \sigma_{AD}, \sigma_D \) are the respective errors in counts for the integrated fluorescence component areas for the acceptor in the presence of donor and donor only. \( \sigma_{\Phi_A}, \sigma_{\Phi_D} \) are the error in the QYs.

\[ \sigma_{EE} = \frac{\Phi_D \phi_{AD}}{\Phi_A \phi_D} \sqrt{\frac{\sigma_D^2}{\phi_D^2} + \frac{\sigma_{AD}^2}{\phi_{AD}^2} + \frac{\sigma_{\Phi_D}^2}{\Phi_D^2} + \frac{\sigma_{\Phi_A}^2}{\Phi_A^2}} \] (4.35)

### 4.9.7 FRET Step Efficiencies

The step efficiencies for the individual energy transfer steps were calculated by collecting fluorescence spectra from devices which were comprised of only the individual dye pairs. Unlabeled DNA strands replaced the original labeled strands. The efficiencies were calculated using Equation (4.36) for all the heteroFRET steps. For the Cy3-Cy3 homoFRET the anisotropy values were used assuming the R6 device represented a complete lack of anisotropy. The efficiencies for the homoFRET step was calculated using Equation (4.38). Where \( F_{DA} \) is the fluorescence component area of the donor in the presence of acceptor, \( F_D \) is the fluorescence component area of the donor in the absence of acceptor.\(^{27}\)

\[ E_D = 1 - \frac{F_{DA}}{F_D} \] (4.36)
Equation (4.36) is can also be expressed as a function of lifetimes as Equation (4.37). Where $\tau_{R1}$, $\tau_{R2}$, and $\tau_{R6}$ are the anisotropies for each R1, R2, and R6 device, respectively. A plot of the individual energy transfer step is shown in Figure 4.6. These values were used in modeling and to better understand the system.

$$E_D = 1 - \frac{F_{DA}}{F_D} = 1 - \frac{\tau_{DA}}{\tau_D}$$ (4.37)

$$E_{Cy3\rightarrowCy3} = \frac{\tau_{R1} - \tau_{R2}}{\tau_{R1} - \tau_{R6}}$$ (4.38)

**Figure 4.17.** A plot of the FRET efficiency for each independent FRET steps for each respective device. The FRET efficiency was calculated using donor quenching, Equation (4.36).
Cy3.5-Cy3 Step Efficiency

Figure 4.18. A plot of the FRET efficiency for each independent FRET steps for each respective device, calculated using Equation (4.38).

4.9.8 Fluorescence Lifetime Anisotropy

The anisotropy decay rates for the full device is faster than the corresponding single arm structures suggesting that the arms of the full structure do interact, and thus present additional homoFRET (and therefore likely HetreoFRET) pathways between arms. The fluorescence lifetime anisotropy decay for R1 stars was found to be slightly higher than the same values for R2 in the free floating arms. This suggests the fluorophores on the arms collectively behave like a single alone acceptor.

Table 4.9. Fluorescence lifetime anisotropy measurements for each device.

<table>
<thead>
<tr>
<th>Device</th>
<th>R1</th>
<th>R2</th>
<th>R6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Arm</td>
<td>5.03±0.66</td>
<td>3.12±0.24</td>
<td>0.43±0.5</td>
</tr>
<tr>
<td>4 Arm Star</td>
<td>3.74±0.38</td>
<td>1.11±0.27</td>
<td>0.67±0.2</td>
</tr>
<tr>
<td>8 Arm Star</td>
<td>3.28±0.31</td>
<td>0.87±0.23</td>
<td>0.69±0.3</td>
</tr>
</tbody>
</table>
Figure 4.19. (a-c) Fluorescence lifetime anisotropy spectra of the full four-arm and eight-arm star R1, R2, and R6 devices vs. the respective single arm devices. d) A plot of the determined anisotropies lifetimes in units of ns.

4.9.9 Antenna Effect

The antenna effect \((AE)\) is defined as Equation (4.39).\textsuperscript{30–32} The \(AE\) is the ratio between the fluorescence intensity of the terminal Cy5.5 acceptor excited at 466 nm in the presence of all the dyes to the direct excitation of the Cy5.5 acceptor at 685 nm. The experimental \(AE\) \((AE_E)\) is defined by Equation (4.40). Where \(I_{Cy5,515\,nm}\), \(I_{Cy5,685\,nm}\), and \(I_{Cy5,635\,nm}\) are the fluorescence intensities of the terminal Cy5.5 following excitation of the initial Cy3 donor at 515 nm and direct excitation at 635 or
685 nm, respectively.

$$AE = \frac{I_{Cy5, 515 \text{ nm}}}{I_{Cy5, 685 \text{ nm}}}$$ \hspace{1cm} (4.39) \hspace{1cm} $$E_{AE} = \frac{I_{Cy5, 515 \text{ nm}}}{I_{Cy5, 635 \text{ nm}}}$$ \hspace{1cm} (4.40)

<table>
<thead>
<tr>
<th>Device (Arm or Branch Length)</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x Cy3</td>
<td>3.8 ± 0.7</td>
<td>4.3 ± 0.2</td>
<td>5.4 ± 0.8</td>
<td>5.5 ± 0.4</td>
<td>5.7 ± 0.3</td>
<td>5.8 ± 0.4</td>
</tr>
<tr>
<td>2x Cy3</td>
<td>7.7 ± 0.7</td>
<td>8.9 ± 0.8</td>
<td>10.0 ± 0.9</td>
<td>10.9 ± 1.1</td>
<td>11.9 ± 1.1</td>
<td>11.8 ± 1.0</td>
</tr>
</tbody>
</table>

4.9.10 Fluorophore Distance Measurements

The average anisotropy was used to calculate the Cy3-Cy3 homoFRET transfer efficiency and distance between dyes since these values are proportional to the steady-state fluorescence intensity.

Table 4.11. The designed and experimentally determined fluorophore distances for each device.

<table>
<thead>
<tr>
<th></th>
<th>Designed $r_{DA}$ (bps)</th>
<th>Designed $r_{DA}$ (nm)</th>
<th>Calculated $R_0$ (nm)</th>
<th>4 Arm Star $r_{DA}$ (nm)</th>
<th>8 Arm Star $r_{DA}$ (nm)</th>
<th>Dendrimer $r_{DA}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF488 - Cy3</td>
<td>9</td>
<td>3.1</td>
<td>6.4</td>
<td>5.0</td>
<td>4.9</td>
<td>4.8</td>
</tr>
<tr>
<td>Cy3 - Cy3</td>
<td>9</td>
<td>3.1</td>
<td>4.5</td>
<td>4.8</td>
<td>4.8</td>
<td>4.8</td>
</tr>
<tr>
<td>Cy3 - Cy3.5</td>
<td>10</td>
<td>3.4</td>
<td>5.3</td>
<td>4.9</td>
<td>5.2</td>
<td>5.3</td>
</tr>
<tr>
<td>Cy3.5 - Cy5</td>
<td>10</td>
<td>3.4</td>
<td>5.9</td>
<td>5.7</td>
<td>5.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Cy5 - Cy5.5</td>
<td>6</td>
<td>2.1</td>
<td>6.5</td>
<td>6.0</td>
<td>5.9</td>
<td>5.7</td>
</tr>
</tbody>
</table>

4.9.11 Fluorescence Lifetime

The fluorescence decay lifetime in all cases decreases rapidly from R1 to R2 and then decreases much slower rate. By adding a longer more kinked dsDNA we remove
rigidity to the dye environment, which most likely account for the decrease in lifetime.

The difference between the four and eight-arm stars and dendrimer is likely not as a result of homoFRET but simply the more flexible four and eight-arm devices. The fluorophore lifetime spectra for each device are shown in Figure 4.20. The calculated fluorescence lifetimes are shown in Table 4.12.

![Figure 4.20.](image)

**Figure 4.20.** The fluorescence lifetimes for the Cy3 in the AF488-Cy5.5 devices. (a-c) The raw fluorescent lifetimes for the four-arm star, eight-arm star, and dendrimer devices. (d) A plot of the determined lifetimes in units of ns.

### Table 4.12. The experimentally determined fluorophore lifetimes for each device.

<table>
<thead>
<tr>
<th>Device</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Arm Star</td>
<td>1.37 ± 0.09</td>
<td>1.11 ± 0.06</td>
<td>1.04 ± 0.07</td>
<td>1.01 ± 0.08</td>
<td>1.01 ± 0.09</td>
<td>1.04 ± 0.02</td>
</tr>
<tr>
<td>8-Arm Star</td>
<td>1.32 ± 0.04</td>
<td>1.14 ± 0.08</td>
<td>1.06 ± 0.08</td>
<td>1.04 ± 0.09</td>
<td>1.05 ± 0.09</td>
<td>1.09 ± 0.02</td>
</tr>
<tr>
<td>Dendrimer</td>
<td>1.59 ± 0.12</td>
<td>1.33 ± 0.11</td>
<td>1.25 ± 0.17</td>
<td>1.25 ± 0.27</td>
<td>1.17 ± 0.29</td>
<td>1.19 ± 0.04</td>
</tr>
</tbody>
</table>
4.9.12 Cy3 Placement Investigations Anisotropy Measurements

Table 4.13. Experimentally determined fluorophore lifetimes for the R6 devices with different Cy3 fluorophore placements.

<table>
<thead>
<tr>
<th>Device</th>
<th>Cy3 in R6 Position</th>
<th>2Cy3 R1 and R6 Positions</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Arm Star</td>
<td>5.84± 0.66</td>
<td>1.96± 0.45</td>
</tr>
<tr>
<td>8 Arm Star</td>
<td>7.65± 0.7</td>
<td>2.24± 0.43</td>
</tr>
<tr>
<td>Dendrimer</td>
<td>3.85± 0.74</td>
<td>2.93± 0.42</td>
</tr>
<tr>
<td>Unbound Cy3 Control</td>
<td>0.99± 0.09</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.21. (a-c) Fluorescence lifetime anisotropy spectra of a single Cy3 in the R6 position closest to the AF488 vs. the 2Cy3 located at R1 and R6 for the four-arm star, eight-arm star, and dendrimer R6 devices, respectively. (d) The fluorescence lifetime anisotropy spectra for a unbound Cy3 fluorophore.
4.9.13  Four-arm Device (Emission Spectra 466 nm)

Figure 4.22.  The averaged fluorescence spectra of the four-arm star device when excited at 466 nm. (a-d) fluorescence spectra of the respective four-arm star devices, AF488-Cy5.5, AF488-Cy5, AF488-Cy3.5, and AF488 Cy3. (e-g) Fluorescence spectra of the respective four-arm star devices, Cy3-Cy5.5, Cy3-Cy5, and Cy3-Cy3.5.
Figure 4.23. The averaged fluorescence spectra of the eight-arm arm star device when excited at 466 nm. (a-d) Fluorescence spectra of the respective eight-arm arm star devices, AF488-Cy5.5, AF488-Cy5, AF488-Cy3.5, and AF488 Cy3. (e-g) Fluorescence spectra of the respective eight-arm arm star devices, Cy3-Cy5.5, Cy3-Cy5, and Cy3-Cy3.5.
4.9.15  Dendrimer Device (Emission Spectra 466 nm)

Figure 4.24.  The averaged fluorescence spectra of the dendrimer device when excited at 466 nm.  (a-d) Fluorescence spectra of the respective dendrimer devices, AF488-Cy5.5, AF488-Cy5, AF488-Cy3.5, and AF488 Cy3.  (e-g) Fluorescence spectra of the respective dendrimer devices, Cy3-Cy5.5, Cy3-Cy5, and Cy3-Cy3.5.
4.9.16 Four and Eight-arm Star Device (Emission Spectra 515 nm)

Figure 4.25. The averaged fluorescence spectra of the four and eight-arm arm devices when excited at 515 nm. (a-c) and (d-f) fluorescence spectra of the respective four and eight-arm arm devices, Cy3-Cy5.5, Cy3-Cy5, and Cy3-Cy3.5.
Figure 4.26. (a-c) The averaged fluorescence spectra of the respective dendrimer devices, Cy3-Cy5.5, Cy3-Cy5, and Cy3-Cy3.5, when excited with 515 nm light.
4.9.18 Emission Spectra 635 nm

Figure 4.27. (a-c) The averaged fluorescence spectra of the Cy3-Cy5.5 four-arm star, eight-arm arm star, and dendrimer devices when excited at 635 nm light.
Figure 4.28. The averaged fluorescence excitation spectra of the four-arm star device at 700 nm. (a-d) The averaged excitation spectra of the respective four-arm star devices, AF488-Cy5.5, AF488-Cy5, AF488-Cy3.5, and AF488 Cy3. (e-g) The averaged excitation spectra of the respective four-arm star devices, Cy3-Cy5.5, Cy3-Cy5, and Cy3-Cy3.5.
Figure 4.29. The averaged fluorescence excitation spectra of the eight-arm arm star device at 700 nm. (a-d) the averaged excitation spectra of the respective eight-arm arm star devices, AF488-Cy5.5, AF488-Cy5, AF488-Cy3.5, and AF488 Cy3. (e-g) The averaged excitation spectra of the respective eight-arm arm star devices, Cy3-Cy5.5, Cy3-Cy5, and Cy3-Cy3.5.
4.9.21  Dendrimer Device (Excitation Spectra 700 nm)

Figure 4.30. The averaged fluorescence excitation spectra of the dendrimer device at 700 nm. (a-d) The averaged excitation spectra of the respective dendrimer devices, AF488-Cy5.5, AF488-Cy5, AF488-Cy3.5, and AF488 Cy3. (e-g) The averaged excitation spectra of the respective dendrimer devices, Cy3-Cy5.5, Cy3-Cy5, and Cy3-Cy3.5.
REFERENCES


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CHAPTER FIVE: CONCLUSION

In this dissertation, DNA origami self-assembly has been used to construct and investigate two classes of nanophotonic devices, namely, plasmonic waveguide arrays and FRET-based photonic devices. Both classes of devices have been built by arranging either functionalized gold particles or DNA labeled fluorophores using DNA self-assembly on DNA supports.

While the work in this dissertation has shown that it is possible to self-assemble and characterize nanoscale waveguides, it is important to understand some of the limitations associated with the overall technique. The optical properties of the AuNPs are strongly dependent on their individual shape and size, their alignment, and periodicity. Variations in shape and size of the AuNP result from their synthesis process. The alignment of the AuNPs is dependent on the linearity of origami, which is dependent on the formation yield of the origami and its design. While we have successfully increased the linearity or persistence length by crosslinking two nanotubes, deviations in linearity were still observed in AFM. Staple strands and attachment strands are sometimes not fully incorporated into the origami resulting in a poorly formed structure or an attachment site with missing docking strand. Additionally, staple-binding locations on the scaffold can be poisoned by a staple slightly different from the designed DNA sequence. Which is
quite common for synthetic DNA synthesis process where staples are only rudimentary purified to remove strands of different length.

The AuNP attachment yield to the DNA origami template also significantly affects the performance of the waveguide since one missing AuNP would result in a nonfunctioning device. One way of improving the formation yield and AuNP attachment would be to purify further or improve current DNA synthesis techniques. The purification techniques generally result in a very low yield and are therefore not practicable for large-scale production. Despite all these limitations, the techniques allowed for the production of many functional plasmonic waveguides. The optical properties of individual waveguides were shown to be in good agreement with the existing model suggesting modeling can be used to further optimize future waveguide designs and to develop plasmonic networks for future information transformation applications.

The light harvesting FRET-based devices investigated showed a remarkably high antenna effect, which is a promising advancement despite their relatively low energy transfer efficiency. The device’s performance is affected by many of the same factors that limit gold nanoparticle waveguide performance. While we have shown the DNA origami can be used to create reconfigurable HomoFRET regions of up to ten Cy3.5 dye repeats, there are some limitations that should be addressed. For example, the calculated end-to-end transfer efficiency for all the FRET-based devices were noticeably lower than the predicted values. Three main factors are believed to produce this difference: (1) incompletely formed structures, (2) unfavorable dye orientations, and (3) dye quenching by the DNA.
DNA self-assembly, while massively parallel, produces a distribution of structures which vary in the quality of their formation. While purification techniques are available in most cases, they result in very low yields and in some cases are destructive to the self-assembled DNA structure. Control over the dipole orientation could increase the efficiency of current FRET-based energy harvesting antennas and photonic wires. However, new fluorophore attachment chemistries need to be developed. Bioconjugation methods enabling the attachment of multiple linkers on the same fluorophore should allow for better control over dipole orientation and open the door for high-efficiency devices.

Even though overall transfer efficiency of the FRET-based wire is still low, it is encouraging considering that energy is being transferred between fluorophores a minimum of 13 times. Realistically, due to the random walk nature of the HomoFRET the number of steps is considerably higher. It is apparent that DNA self-assembly formation efficiency is a common failure mechanism across all of the devices investigated. DNA self-assembly formation efficiency is expected to increase as the field of structural DNA technology evolves through further optimizations in the synthesis of synthetic DNA. It is clear that DNA self-assembled photonic devices have yet to reach their full potential. DNA-templated self-assembly of photonic devices will continue to be a focus of research as the need for transferring information at a reduced wavelength has many current and future applications ranging from biosensing to energy harvesting.
APPENDIX A: AUTHOR PERMISSION LETTERS

Mr. Charles N. Schmidt

Mr. Blake Rapp

Mr. Sadao Takabayashi

Dr. Elton Graugnard

Dr. William B. Knowlton
August 12, 2016

Dear Mr. Charles Schmidt,

I am writing to request permission to use the article, *Multiscaffold DNA Origami Nanoparticle Waveguides*, of which I am the first author, and which appeared in Nano Letters (July 03, 2013, page 13 (8), pp 3850-3856, DOI:10.1021/nl401879r) for which you were a contributing author, in my Boise State University dissertation. I have received written authorization from Nano Letters to include the article and supporting documents as part of my dissertation.

I will include appropriate citations to the work and copyright and reprint rights in the appendices. The bibliographic citation will appear in the References list. Please indicate your approval of this request by signing in the space provided. If you have questions, please call me at the number above.

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Material description: The entire published article and all the supporting documents including figures and tables of the manuscript entitled, *Multiscaffold DNA Origami Nanoparticle Waveguides*.

Signed: ______________________________ Date: 13 Aug 2016
William P. Klein  
Micron School of Materials Science & Engineering, Boise State University  
1910 University Drive MS2090, Boise, ID 83725  
(509) 929-7337

Mr. Blake Rapp  
Micron Technology Inc  
8000 S Federal Way, Boise, ID 83716  
(208) 908-9362

August 12, 2016

Dear Mr. Blake Rapp,

I am writing to request permission to use the article, *Multiscaffold DNA Origami Nanoparticle Waveguides*, of which I am the first author, and which appeared in Nano Letters (July 03, 2013, page 13 (8), pp 3850-3856, DOI:10.1021/nl401879r) for which you were a contributing author, in my Boise State University dissertation. I have received written authorization from Nano Letters to include the article and supporting documents as part of my dissertation.

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Date: 12 Aug 2016
William P. Klein  
Micron School of Materials Science & Engineering, Boise State University  
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Sadao Takabayashi  
Micron School of Materials Science & Engineering, Boise State University  
1910 University Drive MS2090, Boise, ID 83725  
(805) 280-9669

August 12, 2016

Dear Mr. Sadao Takabayashi,

I am writing to request permission to use the article, Multiscaffold DNA Origami Nanoparticle Waveguides, of which I am the first author, and which appeared in Nano Letters (July 03, 2013, page 13 (8), pp 3850-3856, DOI:10.1021/nl401879r) for which you were a contributing author, in my Boise State University dissertation. I have received written authorization from Nano Letters to include the article and supporting documents as part of my dissertation.

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William P. Klein  
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Dr. Elton Graugnard  
Micron School of Materials Science & Engineering, Boise State University  
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October 31, 2016

Dear Dr. Elton Graugnard,

I am writing to request permission to use the article, *Multiscaffold DNA Origami Nanoparticle Waveguides*, of which I am the first author, and which appeared in Nano Letters (July 03, 2013, page 13 (8), pp 3850-3856, DOI:10.1021/nl401879r) for which you were a contributing author, in my Boise State University dissertation. I have received written authorization from Nano Letters to include the article and supporting documents as part of my dissertation.

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Signed: Elton Graugnard Date: 10/31/16
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Dr. William Knowlton  
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August 15, 2016

Dear Dr. William Knowlton,

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