UNIVERSITY OF CALIFORNIA, SAN DIEGO

Extended Fluorescent Resonant Energy Transfer in DNA Constructs

A Dissertation submitted in partial satisfaction of the requirements for the degree
Doctor of Philosophy

in

Materials Science and Engineering

by

Taeseok Oh

Committee in charge:

Professor Michael J. Heller, Chair
Professor Yi Chen
Professor Xiahua Huang
Professor Yu-Hwa Lo
Professor Donald J. Sirbuly

2016
Copyright
Taeseok Oh, 2016
All rights reserved.
The Dissertation of Taeseok Oh is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2016
DEDICATION

To Misun and Alice
EPIGRAPH

Improvements are invented
only by those who can feel that something is not good.

— Friedrich Nietzsche
## TABLE OF CONTENTS

Signature Page ................................................................. iii

Dedication ................................................................. iv

Epigraph ................................................................. v

Table of Contents ........................................................ vi

List of Figures ............................................................ viii

List of Tables .............................................................. xi

Acknowledgements ........................................................ xii

Vita ................................................................. xiv

Abstract of the Dissertation ............................................. xv

| Chapter 1 | Introduction | 1
| 1.1 | Fundamentals of FRET | 1 |
| 1.2 | FRET application with DNA | 4 |
| 1.3 | Contact quenching | 5 |
| 1.3.1 | Quenching by Dimerization [48, 4, 22] | 6 |
| 1.3.2 | Quenching by DNA bases [46] | 8 |
| 1.3.3 | Quenching by oxygen in water molecule [32] | 9 |

| Chapter 2 | Materials | 12 |
| 2.1 | DNA | 12 |
| 2.2 | Fluorophores | 12 |
| 2.3 | Linkers | 13 |
| 2.4 | Surfactant and Micelle | 14 |
| 2.4.1 | Cetyltrimethylammonium Bromide | 14 |
| 2.4.2 | Triton-X 100 | 14 |
| 2.4.3 | Sodium dodecyl sulfate | 15 |

| Chapter 3 | CTAB enhancement of FRET in DNA structures | 17 |
| 3.1 | Introduction | 17 |
| 3.2 | Materials and Methods | 18 |
| 3.3 | Results | 19 |
| 3.3.1 | TAMRA on DNA | 19 |
| 3.3.2 | TexasRed on DNA | 24 |
| 3.3.3 | FRET on DNA | 25 |
| 3.4 | Conclusion | 28 |
Chapter 4 Enhanced Fluorescent Resonant Energy Transfer of DNA Conjugates Complexed with Surfactants and Divalent Metal Ions
4.1 Introduction
4.2 Materials and Methods
4.3 Results
4.4 Conclusion
4.5 Acknowledgement

Chapter 5 Enhancement of fluorescent resonant energy transfer and the antenna effect in DNA structures with multiple fluorescent dyes
5.1 Introduction
5.2 Materials and Methods
5.3 Results
5.3.1 Three TAMRA dye conjugated DNA structures without TexasRed acceptor
5.3.2 Enhanced FRET and Antenna Effect in dsDNA Structures
5.4 Conclusion
5.5 Acknowledgement

Chapter 6 Summary and Future work
6.1 Thesis summary
6.1.1 CTAB enhancement of FRET in DNA structures
6.1.2 Enhanced Fluorescent Resonant Energy Transfer of DNA Conjugates Complexed with Surfactants and Divalent Metal Ions
6.1.3 Enhancement of fluorescent resonant energy transfer and antenna effect in multiple fluorescent dye DNA structures using surfactants and metal ions
6.2 Further Experiments and Future work

Bibliography
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Transmission manager handle received and generated packets. It run on application layer and is connected with set of running applications [13]</td>
<td>3</td>
</tr>
<tr>
<td>1.2</td>
<td>The FRET efficiency as a function of donor and acceptor distance according to the equation 1.3 [9]</td>
<td>3</td>
</tr>
<tr>
<td>1.3</td>
<td>DNA nanostructures provide a useful tool for the organization of photonic components in a linear fashion or in branched networks. [38]</td>
<td>4</td>
</tr>
<tr>
<td>1.4</td>
<td>The space around a donor fluorophore can be divided into four zones. These zones are shown here on a logarithmic scale with the outer radius of each ring being a factor of 10 larger than the inner radius. [38]</td>
<td>6</td>
</tr>
<tr>
<td>1.5</td>
<td>Model of transition dipole interactions in dye H- and J-aggregates and resulting absorption properties [43]</td>
<td>8</td>
</tr>
<tr>
<td>1.6</td>
<td>Absorbance spectrum of trastuzumab conjugated rhodamine fluorophores without SDS (solid line) and with SDS (dashed line). The blue-shifted peak without SDS representes H-dimer formation of fluorophores. [48]</td>
<td>9</td>
</tr>
<tr>
<td>1.7</td>
<td>(a) Change of absorbance spectrum by dimerization of TAMRA dyes caused by oxidation and reduction of disulfate bridge connecting two dyes (b) Time-scale experiment of dimerization shown by the intensity ratio of monomer to dimer in absorbance spectrum. [4]</td>
<td>10</td>
</tr>
<tr>
<td>1.8</td>
<td>Stern-Volmer plots of (A) TAMRA, (B) Alexa 546 and (C) Cy3B [46]</td>
<td>11</td>
</tr>
<tr>
<td>2.1</td>
<td>Chemical structure of the TAMRA donor and TexasRed acceptor.</td>
<td>13</td>
</tr>
<tr>
<td>2.2</td>
<td>Normalized absorbance (Dot line) and emission spectra (solid line) of the TAMRA donor (Blue) and TexasRed acceptor (Red)</td>
<td>13</td>
</tr>
<tr>
<td>2.3</td>
<td>Chemical structure of (a) Thymidine-5-C6 Amino linker, (b) Thymidine-5-C2 Amino linker, and (c) 2’-Deoxyadenosine-8-C6 Amino linker</td>
<td>14</td>
</tr>
<tr>
<td>2.4</td>
<td>Chemical structure of (a) CTAB, (b) Triton X-100, and (c) SDS</td>
<td>16</td>
</tr>
<tr>
<td>3.1</td>
<td>Maximum intensities of fluorescence emissions from (a) TAMRA conjugated on DNA and (b) TexasRed conjugated on DNA as excited at 520 nm</td>
<td>20</td>
</tr>
<tr>
<td>3.2</td>
<td>Size distribution of (a) ssDNA and (b) dsDNA by DLS</td>
<td>21</td>
</tr>
<tr>
<td>3.3</td>
<td>Schematics of the change in the DNA-CTAB complex with increasing CTAB concentration</td>
<td>23</td>
</tr>
<tr>
<td>3.4</td>
<td>Wavelength change of maximum peak in emission spectrum with respect to CTAB concentration</td>
<td>26</td>
</tr>
<tr>
<td>3.5</td>
<td>Fluorescence emission spectra of TAMRA, TexasRed and hybridized FRET probe with (a) no CTAB and (b) 50 uM of CTAB. (c) Maximum emission intensities of each dye in FRET probe extracted from overall emission spectra in the hybrid (Strand Ia+IIa) as excited at 520 nm</td>
<td>27</td>
</tr>
<tr>
<td>3.6</td>
<td>Time-dependent changes of maximum intensities of TexasRed emission in the FRET probe</td>
<td>29</td>
</tr>
</tbody>
</table>
Figure 4.1: (a) Fluorescence emission spectrum excited at 520 nm and (b) Absorbance spectra of TAMRA and TexasRed conjugated ssDNA and dsDNA and FRET hybrid (C6-linked or C2-linked TAMRA donor hybrid) 36

Figure 4.2: Absorbance spectra of (a) C6-linked TAMRA, (b) C2-linked TAMRA, and (c) TexasRed acceptor conjugating on dsDNA .......................... 37

Figure 4.3: Schematics on (a) quenched emission by dimerization without surfactant and (b) enhanced emission by FRET with surfactant in dye-conjugated DNA ............................... 37

Figure 4.4: (a) Emission intensity of TexasRed acceptor and (b) absorbance peak ratio of the TexasRed acceptors monomer to TAMRA donors dimer based on Figure 4.5 and 4.6 with respect to different concentration of surfactants and cations ........................................ 38

Figure 4.5: Absorbance spectra of C6-linked TAMRA on DNA FRET system with different concentration of (a) Triton X-100, (b) CTAB, (c) SDS, (d) sodium with 10 mM SDS and (e) magnesium with 10 mM SDS .......................... 39

Figure 4.6: Absorbance spectra of C2-linked TAMRA on DNA FRET system with different concentration of (a) Triton X-100, (b) CTAB, (c) SDS, (d) sodium with 10 mM SDS and (e) magnesium with 10 mM SDS .................. 40

Figure 4.7: (a) Emission spectrum and (b) Absorbance spectum of TAMRA donor (black bold), TexasRed acceptor on ssDNA, pristine FRET, FRET with 10 mM of Triton X-100, CTAB, SDS, SDS with 50 mM NaCl and SDS with 50 mM MgCl$_2$ in C6 linker based FRET system .......................... 42

Figure 4.8: (a) Emission spectrum and (b) Absorbance spectum of TAMRA donor (black bold), TexasRed acceptor on ssDNA, pristine FRET, FRET with 10 mM of Triton X-100, CTAB, SDS, SDS with 50 mM NaCl and SDS with 50 mM MgCl$_2$ in C2 linker based FRET system .......................... 42

Figure 5.1: Organization of photosystems in the thylakoid membrane [41] .......................... 46

Figure 5.2: Schematics representation for: (a) three TAMRA on ss-DNA and ds-DNA structures without the presence of the TexasRed dye and (b) three TAMRA on ds-DNA structures with TexasRed acceptor dye present in the complementary sequence ................................. 51

Figure 5.3: (a) Maximum intensity in emission spectra and (b) the monomer ratio in absorbance spectra of control sequence A (blank gray), B (blank black), control hybrid C (filled gray) and D (filled black) .............................. 55

Figure 5.4: Absorbance spectra of (a) 3 base spaced three TAMRA on ssDNA, (b) 3 base spaced three TAMRA on dsDNA, (c) 7 base spaced three TAMRA on ssDNA and (d) 7 base spaced three TAMRA on dsDNA with respect to surfactant and cation conditions ........................................ 57

Figure 5.5: Antenna effect values for FRET dsDNA hybrid structures ............................... 59

Figure 5.6: Maximum intensity of donor and acceptor by decomposition of overall emission spectrum in FRET hybrid E, F, G and H as excited at 565 nm (Inset: maximum value among emission intensity from acceptor in each surfactant condition) ............................. 60
Figure 5.7:  Fluorescence emission spectrum of the Texas Red acceptor in FRET dsDNA hybrids (E, F, G, H) with and without 10mM SDS with 50mM Mg$^{2+}$ ion. ..................................................... 64

Figure 6.1:  Schematics of three TAMRA donors and one TexasRed acceptor on ds-DNA structures with/without SDS micelle and cations .................. 70

Figure 6.2:  Schematics of five TAMRA donors and one TexasRed acceptor on ds-DNA structures with/without SDS micelle and cations .................. 71
LIST OF TABLES

Table 3.1: DNA Sequences and conjugated Dye ........................................... 19
Table 3.2: Zeta potential (mV) of complex of CTAB and Dye conjugated DNA .. 23
Table 4.1: Information on dye conjugated DNA (Chemical structure of the linkers is shown in Fig. S1) ................................................................. 34
Table 5.1: DNA sequences and the positions of donor and acceptor dyes .......... 52
ACKNOWLEDGEMENTS

First and foremost I would like to thank my advisor Dr. Michael J. Heller. It has been an honor to study in his research group. I appreciate his guidance and support not only in my research but also in my PhD life. I appreciate all his contributions of advise, time, research idea, and financial support to make my Ph.D experience productive. The joy and enthusiasm he has for the research was contagious and motivational for me, even during tough times in my Ph.D pursuit.

I would like to thanks the committee members, Professor Yi Chen, Professor Xiahua Huang, Professor Yu-Hwa Lo, and Professor Donald J. Sirbuly. Their advice and comments helped keep me motivated in my research.

I would like to thank my parents and parents-in-law for all they have done for me. Also, I appreciate all families including my sister, sister-in-law and brother-in-law encouragin and supporting me.

Finally, I sincerely thank my wife Misun. Your smile always enables me to endure and keep forwarding in struggled moments. I appreciate you sharing joy and sorrow with me. I am proud of myself being your husband. Also, our little special present, Alice. I thank your existence warms my heart.

Chapter 3 is a reprint of the material as it appears in ”CTAB enhancement of FRET in DNA structures, Journal of Biophotonics”, by Taeseok Oh, Tsukasa Takahashi, Sejung Kim, Michael J. Heller, Journal of Biophotonics, 2016. The dissertation/thesis author was the primary investigator and author of this paper.

Chapter 4 is a reprint of the material as it appears in ”Enhanced Fluorescent Resonant Energy Transfer of DNA Conjugates Complexed with Surfactants and Divalent Metal Ion’s”, by Taeseok Oh, Jae-young Choi and Michael J. Heller, Analyst, 2016. The dissertation/thesis author was the primary investigator and author of this paper.

Chapter 5 is a reprint of the material as it appears in ”Enhancement of fluorescent
resonant energy transfer and the antenna effect in DNA structures with multiple fluorescent dyes”, by Taeseok Oh, Sejung Kim, Jae-young Choi, Haeun Chang and Michael J. Heller, Small (submitted), 2016. The dissertation/thesis author was the primary investigator and author of this paper.
VITA

2006 B. S. in Physics, Yonsei University, South Korea
2008 M. S in Mechanical Engineering, Yonsei University, South Korea
2016 Ph. D. in Materials Science and Engineering, University of California, San Diego

PUBLICATIONS

T. Oh, J. Choi, M. J. Heller, Enhanced Fluorescent Resonant Energy Transfer of DNA Conjugates Complexed with Surfactants and Divalent Metal Ions, Analyst, 2016, 141, 2371-2375

T. Oh, T. Takahashi, S. Kim, M. J. Heller, CTAB enhancement of FRET in DNA structures, Journal of Biophotonics, 2016, 9 (1), 49-54


H. Kim, T. Oh, D. Kim, Comparison of Indentation and Scribing Behaviors of Crystalline and Initially Deformed Silicon Tips by Molecular Dynamics Simulation, IEEE Transactions on Magnetics, 2009, 45 (5), 2328-2331
ABSTRACT OF THE DISSERTATION

Extended Fluorescent Resonant Energy Transfer in DNA Constructs

by

Taeseok Oh

Doctor of Philosophy in Materials Science and Engineering

University of California, San Diego, 2016

Professor Michael J. Heller, Chair

This study investigates the use of surfactants and metal cations for the enhancement of long range fluorescent resonant energy transfer (FRET) and the antenna effect in DNA structures with multiple fluorescent dyes. Double-stranded (ds) DNA structures were formed by hybridization of 21mer DNA oligonucleotides with different arrangements of three fluorescent TAMRA donor dyes with two different complementary 21mer oligonucleotides with one fluorescent TexasRed acceptor dye. In such DNA structures, hydrophobic interactions between the fluorescent dyes in close proximity produces dimerization which along with other quenching mechanisms leads to significant reduction of fluorescent emission properties. Addition of the surfactants Triton X-100, cetyltrimethyl ammonium bromide (CTAB) and
sodium dodecyl sulfate (SDS) along with sodium cations (Na\(^+\)) and divalent magnesium cations (Mg\(^{2+}\)) were tested for their ability to reduce quenching of the fluorescent dyes and improve overall fluorescent emission, the long range FRET and the antenna effect properties. When the neutral (uncharged) surfactant Triton X-100 was added to the FRET ds-DNA hybrid structures with three TAMRA donors and one TexasRed acceptor, dye dimerization and emission quenching remained unaffected. However, for the positively charged CTAB surfactant at concentrations of 100 uM or higher, the neutralization of the negatively charged ds-DNA backbone by the cationic surfactant micelles was found to reduce TAMRA dye dimerization and emission quenching and improve TexasRed quantum yield, resulting in much higher FRET efficiencies and an enhanced antenna effect. This improvement is likely due to the CTAB molecules covering or sheathing the fluorescent donor and acceptor dyes which breaks up the dimerized dye complexes and prevents further quenching from interactions with water molecules and guanine bases in the DNA structure. While the negatively charged SDS surfactant alone was not able to reduce dimerization and emission quenching due to repulsive forces between DNA and SDS micelles, the addition of cations such as sodium ions (Na\(^+\)) and divalent magnesium ions (Mg\(^{2+}\)) did lead to a significant reduction in the dimerization and emission quenching resulting in much higher FRET efficiency and an enhanced antenna effect. It appears that when the repulsive electrostatic forces are screened by the cations (Mg\(^{2+}\) in particular), the SDS micelles can approach the FRET ds-DNA structures thereby sheathing or insulating the TAMRA and TexasRed dyes. Overall, the study provides a viable strategy for using combinations of surfactants and cations to reduce adverse fluorescent dye and other quenching mechanisms and improve the overall long distance FRET efficiency and the antenna effect in DNA structures with multi-donor and single acceptor fluorescent dye groups.
Chapter 1

Introduction

1.1 Fundamentals of FRET

Fluorescence resonance energy transfer (FRET) or Förster resonance energy transfer (FRET) is a mechanism describing energy transfer between the isolated donor D and the acceptor A of suitable spectroscopic properties. As the photon does not appear during the energy transfer process, the non-radiative transfer of electron excitation from an excited donor to a ground state acceptor molecule results of the long-range interactions between donor and acceptor dipoles. The FRET also occurs on time scales from femtoseconds to milliseconds at distances ranging from a few to approximately 10 nm.

To observe FRET, the following conditions must be met:
1) Donor and acceptor must have strong electronic transitions in the UV, visible, or IR.
2) Spectral overlap must exist between donor emission and acceptor absorbance.
3) Donor and acceptor must be close, but not too close.
4) The orientation factor should not be too small.
5) The donor emission should have a reasonably high quantum yield.
\[ k_{ET} = \frac{9000 \ln(10) \kappa^2 \Phi_D}{128 \pi^3 n^2 N_A \tau_D R^6} = \int_0^\infty \frac{F_D(\nu) \varepsilon_A(\nu)}{\nu^4} d\nu \]  

(1.1)

\[ k_{ET} = \frac{1}{\tau_D} \left( \frac{R_0}{R} \right)^6 \]  

(1.2)

\[ E = \frac{k_{ET}}{k_{ET} + 1/\tau_D} = \frac{R_0^6}{R_0^6 + R^6} = \frac{1}{1 + (R/R_0)^6} \]  

(1.3)

\[ E = \frac{F_D - F_{DA}}{F_D} = 1 - \frac{F_{DA}}{F_D} \]  

(1.4)

\[ E = \frac{\tau_D - \tau_{DA}}{\tau_D} = 1 - \frac{\tau_{DA}}{\tau_D} \]  

(1.5)

The rate constant for dipole-dipole resonance energy transfer is expressed by equation 1.1 which is derived by Förster (1948, 1968). For the calculation, the measurable spectroscopic quantities are required such as the refractive index of the medium, \( n \); the orientation factor, \( \kappa^2 \) which is typically assumed to be 2/3 for random orientations (Dale et al., 1979); the fluorescence quantum yield of the donor, \( \Phi_D \); the fluorescence lifetime of the donor, \( \tau_D \); Avogadro’s number, \( N_A \); the normalized fluorescence spectrum of the donor, \( F_D(\nu) \); the absorption spectrum of the acceptor, expressed by its extinction coefficient, \( \varepsilon_A(\nu) \); and the average transition frequency \( \nu \) in cm\(^{-1}\). The equation 1.1 can be also expressed to the equation 1.2 in terms of the Forster critical transfer radius \( R_0 \), the distance at which the energy transfer efficiency becomes equal to 50 percent. The efficiency of FRET, \( E \) is then defined as the equation 1.3 as a function of donor and acceptor distance as shown in figure 1.2. The sixth-power distance dependence of FRET has results in its wide-spread
Figure 1.1: Transmission manager handle received and generated packets. It run on application layer and is connected with set of running applications [13].

Applications to measure separations between donor and acceptor molecules. The efficiency is also measured using the relative fluorescence intensity of the donor (Equation 1.4) or fluorescence lifetime of the donor (Equation 1.5), in the absence ($F_D, \tau_D$) and presence ($F_{DA}, \tau_{DA}$) of the acceptor.

Figure 1.2: The FRET efficiency as a function of donor and acceptor distance according to the equation 1.3. $R_0$ is the distance at which the loss of excited state energy by the donor via FRET or fluorescence emission are equally likely. Efficiency varies with the inverse 6th power of the distance between donor and acceptor, and is negligible beyond $2R_0$. It is this latter fact which makes FRET such a powerful indicator of molecular proximity well below the classical optical resolution limit of microscopes [9].
Figure 1.3: DNA nanostructures provide a useful tool for the organization of photonic components in a linear fashion or in branched networks. The modularity of assembly, along with the plethora of DNA functionalization of photonic components, allows for the construction of photonic molecular circuits. Light-harvesting complexes can be spatially clustered and aligned, where sequential energy or charge-transfer processes lead to optimized channelling efficiency, to create a new generation of photonic wires, plasmonic or conducting devices (blue, green and red spheres and orange rods represent photonic components that can serve as light-harvesting and energy-transfer materials). Enzymes or membrane complexes (uneven green spheres) can be used as final energy or electron acceptors, acting as molecular transducer units, where light is transformed into chemical potential (represented by the transformation of substrate (triangles) into a higher-energy product (stars)). Physical separation of photonic components creates a new layer of spectral separation, allowing the construction of larger and more complex photonic circuitry [38]

1.2 FRET application with DNA

Photosynthesis, the basis of all life on Earth, boasts the remarkable ability to transform solar energy into chemical energy, and has driven chemists to design artificial systems that mimic its every aspect [36]. In particular, supramolecular chemistry has contributed greatly to the design of artificial light harvesting, energy transfer and charge separation complexes [64, 3]. The main drawback to traditional approaches is the need for extensive organic synthesis efforts, leading to two extreme situations: small constructs with two to five functional units and sub-nanometer level spatial control, or longer constructs with many repeating units, but reduced control over the overall shape and size. The bottom-up assembly of organic suprastructures affords spatial control at the sub-nano level; DNA nanostructures can be used as the interface between molecular entities to provide nanometre-scale precise junctions to attach different molecular entities. For example, light-harvesting complexes
can be put in close contact with charge transfer units in a modular fashion, using DNA as a molecular pegboard. This might constitute a fresh approach for the construction of artificial leaf systems (Figure 1.3). The often water-insoluble systems can be placed side by side with proteins or other biomolecules. Furthermore, increasing knowledge of the functionalization of nanoparticles with oligonucleotides encourages the use of DNA nanostructures as motherboards for many potential applications. The ability of DNA to transport charge over considerable distances along its bases is a consequence of oxidation [14, 51], a process that can compromise the integrity of the strand or even the structure. Thus, double-stranded DNA [12, 59, 57] and DNA orgami [55] have been used as scaffolds for dye-based photonic wires, where energy is transferred in a linear fashion over tens of nanometres. DNA nanostructures are intrinsically more rigid than double-stranded DNA and can be used to build longer photonic wires, and further, the unique 2D and 3D spatial arrangements allow the construction of branched paths for energy transfer. Combining plasmonic nanostructures [58], semiconductors and proteins in complex networks leads to the concept of molecular circuits, where photons and chemical and electrical potential can be interconverted. DNA boards may also be used to merge bottom-up and top-down methods for the organization of chemically synthesized inorganic nanowires [65].

### 1.3 Contact quenching

Förster visualized a donor as a group of electrical oscillators close together. These electrical oscillators produce an electrical field in the space around the donor. This space consists of four zones: the contact zone or Dexter zone [5], the near zone or the near field, the intermediate zone, and the far zone (also called the far-field or the radiation zone). The concept of zones, illustrated in Figure 1.4, dates back to Hertz [37] who actually considered three zones: the near, intermediate, and far, because he set out to confirm Maxwells prediction of electromagnetic waves [37] and was not interested in distances very
Figure 1.4: The space around a donor fluorophore can be divided into four zones. These zones are shown here on a logarithmic scale with the outer radius of each ring being a factor of 10 larger than the inner radius. The donor occupies the center of the contact zone, which extends up to about a nanometer or more depending on the donor size. Around this zone is the near field, about 110nm from the donor. The near field is the only zone where Förster theory applies. FRET happens in the near field, that is, roughly in the 110nm range. Although FRET efficiency is depending on a function of donor and acceptor distance as shown in Figure 1.2. If it is less than about 1 nm, Förster theory does not apply for at least two reasons: First, the complex formation may occur between donor and acceptor at such a proximity, which is a contact quenching. Second, the Försters theory is based on the ideal dipole approximation (IDA) and the IDA breaks down if the donor-acceptor distance is on the order of 1nm. The contact quenching (also referred to as static quenching) can dominantly occur when the molecules form a complex in the ground state, i.e. before excitation occurs. The complex has its own unique properties, such as being nonfluorescent and having a unique absorption spectrum.

1.3.1 Quenching by Dimerization [48, 4, 22]

Dye aggregation is well-known and is often attributed to hydrophobic effects - the dye molecules stack together to minimize contact with water. Planar aromatic dyes that are matched for association through hydrophobic forces can enhance static quenching. Steric
and electrostatic forces may also determine if, and how, dyes aggregate [28]. Traditionally, dye aggregates are classified as H- and J-type on the basis of the observed spectral shift of the absorption maximum relative to the respective monomer absorption band (hypsochromic for H-type and bathochromic for J-type). Many J-aggregates exhibit fluorescence, and their fluorescence quantum yield quite often surpasses that of the monomeric dyes [53, 50]. In contrast, it is well documented that the fluorescence of H-aggregates is strongly quenched. This behavior was already observed a long time ago for a large number of dimer aggregates of classical fluorophores, including fluorescein, eosin, thionine, methylene blue, and certain cyanine dyes, and the non-emissive character of the excited state became commonly accepted as a general feature of H-aggregates. Theoretical interpretation by Förster (coupled oscillator model) and Kasha (exciton theory) could plausibly explain the nonfluorescent nature of dimeric as well as extended H-type aggregates [25, 10]. As shown in Figure 1.5, two exciton states arise in the case of face-to-face-stacked dimer aggregates, but only the transition to the higher energy exciton state is allowed, and can be observed in the UV/Vis absorption spectrum as a blue-shifted band. Subsequent rapid internal conversion of this excited state into the lower energy exciton state quenches the fluorescence as a result of the decreasing transition probability for a radiative process from this state to the ground state.

In homodimers, in which two of the same dye form a ground-state complex, it is possible for an H-type dimer to be totally nonfluorescent if the dyes align such that their identical transition dipole moments completely cancel. However, in heterodimers, when both individual dyes are fluorescent (e.g., rhodamine and fluorescein), the H-type dimer will only have diminished fluorescence because it is very unlikely for the transition dipole moments to completely cancel. Nevertheless, in the case of a heterodimer between a fluorophore and a dark quencher, coupling of the fluorophore’s excitation to the quencher’s dark channel can make the ground-state complex completely nonfluorescent. Some fluorophores, such as rhodamine and BODIPY derivatives, form homodimers at relatively high local
Figure 1.5: Model of transition dipole interactions in dye H- and J-aggregates and resulting absorption properties. The ovals correspond to the molecular profile; the double arrow indicates the polarization axis for the molecular electronic transition considered; $S_0$ and $S_1$ are the electronic levels whereas $S_1^+$ and $S_1^-$ represent the exciton states of the composite molecule. [43]

concentrations, and the photophysical chemistry underlying quenching by dimerization in concentrated aqueous solutions has been well studied [1, 27].

Experimentally, H-dimer formation is typically observed by blue-shifted peak in absorbance spectrum (Figure 1.6. Based on change of absorbance intensity of the blue-shifted dimer peak and the original monomer peak, the degree of fluorophore’s dimerization could be estimated by using UV/Vis absorbance spectrometer. In terms of Tetramethylrhodamine (TAMRA) which is discussing in this thesis, dimer and monomer peak in absorbance spectrum is corresponding to 520 nm and 553 555 nm, respectively. For example of the absorbance ratio change induced by dimerization, the Figure 1.7 shows that decreasing absorbance ratio indicates the dimerization in the experiment.

1.3.2 Quenching by DNA bases [46]

The characterization of dye-DNA interactions is important in biophysical research not only because they play an important role in the interpretation of FRET experiments, but also because they can potentially affect the structure and dynamics of the biopolymer itself. The interactions of fluorescent dyes with nucleotides have been extensively studied for a large
variety of coumarins, rhodamines, xanthenes and cyanines by means of fluorescence quenching experiments [6, 7, 17, 52, 61]. Depending on the electrochemical potentials of their singlet excited states, electron transfer from or to the nucleotide can occur, resulting in the formation of the radical anion or cation of the dye. Most commonly, quenching occurs by nucleotide oxidation and is most efficient for deoxyguanosine monophosphate, which has the lowest oxidation potential among the four DNA bases [52, 66, 23]. As the photo-induced electron transfer can quench many fluorophores including TAMRA which is discussed in this thesis, Figure 1.8 shows that presence of DNA bases can highly affect the fluorescence emission of TAMRA.

1.3.3 Quenching by oxygen in water molecule [32]

One of the best-known collision quenchers is molecular oxygen [26], which quenches almost all known fluorophores. Depending upon the sample under investigation, it is frequently necessary to remove dissolved oxygen to obtain reliable measurements of the
Figure 1.7: (a) Change of absorbance spectrum by dimerization of TAMRA dyes caused by oxidation and reduction of disulfate bridge connecting two dyes: Once dimer forms favorably by oxidation, the absorbance intensity of dimer peak (520 nm) increases in absorbance spectrum. In contrast, when reduction occurs, monomer peak (553-555 nm) increases as the dimer peak decreases. (b) Time-scale experiment of dimerization shown by the intensity ratio of monomer to dimer in absorbance spectrum. [4]

fluorescence yields or lifetimes. The mechanism by which oxygen quenches has been a subject of debate. The most likely mechanism is that the paramagnetic oxygen causes the fluorophore to undergo intersystem crossing to the triplet state. In fluid solutions the long-lived triplets are completely quenched, so that phosphorescence is not observed. Aromatic and aliphatic amines are also efficient quenchers of most unsubstituted aromatic hydrocarbons. For example, anthracene fluorescence is effectively quenched by diethylaniline [29]. For anthracene and diethylaniline the mechanism of quenching is the formation of an excited charge-transfer complex. The excited-state fluorophore accepts an electron from the amine. In nonpolar solvents fluorescence from the excited charge-transfer complex (exciplex) is frequently observed, and one may regard this process as an excited state reaction rather than quenching. In polar solvents the exciplex emission is often quenched, so that the fluorophoreamine interaction appears to be that of simple quenching.
Figure 1.8: Stern-Volmer plots of (A) TAMRA, (B) Alexa 546 and (C) Cy3B. The ratio of the fluorescence intensity in the absence ($F_0$) and presence ($F$) of nucleotides is plotted as a function of increasing concentration of deoxynucleoside monophosphates such as dGMP (open squares), dAMP (filled circles), dCMP (open triangles) and dTMP (open circles). Insets: Dynamic Stern-Volmer plot using the quencher-dependent lifetime of the dye as a function of increasing concentrations of dGMP. [46]
Chapter 2

Materials

2.1 DNA

The dye-conjugated and non-conjugated 21mer oligonucleotide single strands were supplied by TriLink BioTechnologies, Inc. Detail DNA information such as DNA sequences, hybridization and concentration is described in the experimental section in each chapter.

2.2 Fluorophores

There are many rhodamine derivatives used for various applications such as FRET and imaging purposes. In this research, carboxytetramethylrhodamine (TAMRA) and sulforhodamine 101 acid chloride (Texas Red) are applied to the donor and acceptor fluorophores, respectively.

Sulforhodamine 101 acid chloride (Texas Red) is a red fluorescent dye, used in histology for staining cell specimens, for sorting cells with fluorescent-activated cell sorting machines, in fluorescence microscopy applications, and in immunohistochemistry [60].

Texas Red fluoresces at about 615 nm, and the peak of its absorption spectrum is at 589 nm. The powder is dark purple. Solutions can be excited by a dye laser tuned to
Figure 2.1: Chemical structure of the TAMRA donor and TexasRed acceptor

Figure 2.2: Normalized absorbance (Dot line) and emission spectra (solid line) of the TAMRA donor (Blue) and TexasRed acceptor (Red)

595 605 nm, or less efficiently a krypton laser at 567 nm.

2.3 Linkers

The fluorescent donor dye TAMRA (Carboxytetramethylrhodamine) is attached to the thymine base in the donor DNA sequences using a C6-linker (six methylene groups) or a C2-linker (two methylene groups), and the fluorescent acceptor dye TexasRed (sulforodamine) was attached to the adenine base located at the middle of acceptor (complementary) DNA strand using a C6-linker (six methylene groups) (Figure 2.3).
2.4 Surfactant and Micelle

2.4.1 Cetyltrimethylammonium Bromide

Cetyltrimethylammonium Bromide [ (C_{16}H_{33})N(CH_{3})_{3}Br ; Cetrimonium bromide; hexadecyltrimethylammonium bromide; CTAB] is a quaternary ammonium surfactant. As with most surfactants, CTAB forms micelles in aqueous solutions. At room temperature, it forms micelles with aggregation number 75-120 (depending on method of determination; average 95) and degree of ionization, $\alpha = 0.2$ (fractional charge; from low to high concentration). The binding constant ($K^0$) of Br- counterion to a CTA$^+$ micelle at room temperature is 400 M$^{-1}$. This value is calculated from Br$^-$ and CTA$^+$ ion selective electrode measurements and conductometry data by using literature data for micelle size ($r = 3$ nm), extrapolated to the critical micelle concentration of 1 mM. However, $K^0$ varies with total surfactant concentration so it is extrapolated to the point at which micelle concentration is zero.

2.4.2 Triton-X 100

Triton X-100 (C_{14}H_{22}O(C_{2}H_{4}O)_{n}) is a nonionic surfactant that has a hydrophilic polyethylene oxide chain (on average it has 9.5 ethylene oxide units) and an aromatic hydrocarbon lipophilic or hydrophobic group. The hydrocarbon group is a 4-(1,1,3,3-tetramethylbutyl)-phenyl group. Undiluted Triton X-100 is a clear viscous fluid (but less
viscous than undiluted glycerol) owing to the hydrogen bonding of its hydrophilic polyethylene oxide parts. It has a viscosity of about 270 centipoise at 25 °C which comes down to about 80 centipoise at 50 °C. Triton X-100 is soluble at 25 °C in water, toluene, xylene, trichloroethylene, ethylene glycol, ethyl ether, ethyl alcohol, isopropyl alcohol, ethylene dichloride, but unless a coupling agent like oleic acid is used, Triton X-100 is insoluble in kerosene, mineral spirits, and naphtha.

2.4.3 Sodium dodecyl sulfate

Sodium dodecyl sulfate (SDS) is a synthetic organic compound with the formula CH₃(CH₂)₁₁SO₄Na. It is an anionic surfactant used in many cleaning and hygiene products. The sodium salt is of an organosulfate class of organics. It consists of a 12-carbon tail attached to a sulfate group. It is the sodium salt of dodecyl hydrogen sulfate, the ester of dodecyl alcohol and sulfuric acid. Its hydrocarbon tail combined with a polar "headgroup" give the compound amphiphilic properties and so make it useful as a detergent. Also derived as a component of mixtures produced from inexpensive coconut and palm oils, SDS is a common component of many domestic cleaning, personal hygiene and cosmetic, pharmaceutical, and food products, as well as of industrial and commercial cleaning and product formulations.
Figure 2.4: Chemical structure of (a) CTAB, (b) Triton X-100, and (c) SDS
Chapter 3

CTAB enhancement of FRET in DNA structures

3.1  Introduction

Fluorescence resonance energy transfer (FRET) has been widely used to study the structure and dynamic properties of biomolecules [20, 56]. Specifically, by using fluorescent dyes conjugated on DNA strands, FRET has been applied to molecular sensors in which fluorescent signals change as a result of hybridization or enzymatic reactions, as well as to photonic wire structures in which photonic energy can transfer along a DNA strand [67, 16, 8, 18]. However, because fluorescence is highly influenced by environmental conditions and surrounding molecules, fluorescence energy transfer from donor dyes to the acceptor dye conjugated on a DNA strand is easily quenched by interactions with the DNA bases, water molecules and other mechanisms which can significantly lower the FRET efficiency [30, 62]. It is also important to note that the fluorescence emission from dyes conjugated on a DNA strand can be easily quenched by fluorescent donor dye interactions and by contact quenching between a donor and the acceptor dye. Additionally, it has been reported in other studies, that electron-transfer from a DNA base to the excited singlet state
of a dye can also quench the fluorescence emission [6, 52, 61]. This quenching is most efficient for guanine, which has the lowest oxidation potential among the four DNA bases [17, 46]. Other common contact quenching, also referred to as static quenching, can occur when dyes are aggregated due to hydrophobic effects in which the dye molecules stack together to minimize contact with water [34, 32].

In this study, we investigated the effects of the cation surfactant cetyl-trimethyl ammonium bromide (CTAB) on reducing quenching and enhancing FRET in hybridized complementary 21mer oligonucleotides, where one DNA strand is conjugated with the donor group 5-Carboxytetramethylrhodamine (TAMRA) and the other with the acceptor group sulforhodamine acid chloride (TexasRed). Possible mechanisms for the enhancement of FRET efficiency between the two dyes were postulated by investigating the fluorescent behavior of the dye conjugated on DNA in CTAB solutions of different concentrations.

3.2 Materials and Methods

The dye-conjugated and non-conjugated 21mer oligonucleotide single strands were prepared as shown in the Table 3.1. In addition to the two non-conjugated complementary DNA strands (strand I and II), the dye conjugated DNA strands contained a fluorescent donor dye (TAMRA) with a hydrocarbon linker (C6) attached on the thymine base located at middle of strand (Ia), and a fluorescent acceptor dye (TexasRed) with a C6 linker attached on the adenine base located at the middle of strand (IIa) (Figure 2.1). The concentrations of the DNA strands in solution were determined by the extinction coefficient and the optical density values using the UV-Vis absorbance measurement. For the dye conjugated ssDNA experiments, 150 nM of strand Ia and strand IIa were prepared in 1X PBS. Also, for preparing the duplex oligonucleotides, 150 nM of the dye conjugated DNA (strand Ia or IIa) was mixed with 150 nM of the non-conjugated complementary strand (strand I or II), then heated to 60°C and cooled down slowly to room temperature for 2 hours. All
experiments were performed by using 1X PBS at pH 7.4 at 20°C. All fluorescence emission measurements were repeated 3 times to ensure a reliable result. CTAB was obtained from Sigma Aldrich and the solution was prepared by dissolving the required amount in a known volume of the buffer solution.

3.3 Results

3.3.1 TAMRA on DNA

The fluorescence intensities were scanned for strands Ia and IIa individually, with the addition of complementary strands I and II, and variable concentrations of CTAB for each condition. All dyeconjugated DNAs were excited at 520 nm and emissions were measured from 550 nm to 700 nm. For FRET tests with two dyes present, in order to quantitatively extract two different emission intensities of the acceptor and donor from the overlapped spectra, pure TAMRA and TexasRed intensities were obtained by a spectrum decomposition method in which the pure TAMRA spectrum was extracted based on normalizing the 560 nm emission intensity of pristine TAMRA and the pure TexasRed spectrum was obtained by subtracting the extracted TAMRA spectrum from the original spectra. In addition, to investigate DNA-CTAB aggregation in the solution, dynamic light scattering (DLS) was used to measure the surface charge and size distribution of the aggregation. The experimental details are shown in the supporting information

<table>
<thead>
<tr>
<th>DNA</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strand I</td>
<td>5’-TGT GTG TGT GTT TTT TGG TTT- 3’</td>
</tr>
<tr>
<td>Strand Ia</td>
<td>5’-TGT GTG TGT G (5-TAMRA)(dT-C6-NH) T TTT TGG TTT-3’</td>
</tr>
<tr>
<td>Strand II</td>
<td>5’-AAA CCA AAA AAC ACA CAC ACA-3’</td>
</tr>
<tr>
<td>Strand IIa</td>
<td>5’-AAA CCA AAA A (TexasRed-X)(dA-C6-NH) C ACA CAC ACA-3’</td>
</tr>
</tbody>
</table>
Figure 3.1: Maximum intensities of fluorescence emissions from (a) TAMRA conjugated on DNA and (b) TexasRed conjugated on DNA as excited at 520 nm.
Figure 3.2: Size distribution of (a) ssDNA and (b) dsDNA by DLS
The emission intensity of TAMRA on the dsDNA (strand Ia+II) was much higher compared to TAMRA on ssDNA in absence of CTAB (Figure 3.1a). Similar to previous studies, the emission intensity of TAMRA conjugated on the ssDNA was quenched by free guanine bases in DNA [17, 46]. Therefore, hybridization reduces the quenching by the free bases and recovers the fluorescent emission from the TAMRA. As the CTAB concentration increased to 25 uM, the emission intensities of TAMRA decreased due to the less polar environment. Below the critical micelle concentration (CMC), which is 200 uM in 1X PBS, CTAB molecules prefer to disperse freely in solution, decreasing the polarity of the solution [2]. It is known that Rhodamine type dyes such as TexasRed and TAMRA are highly fluorescent in polar solvents since a high quantum yield is maintained by restricting the rotation of the phenyl rings in Rhodamine dyes by a bridging oxygen atom [35]. Thus, as the hydrophobic CTAB molecules cause the aqueous solution to be less polar, emission intensities of the dyes decrease in the solution. Also, electrostatic interaction between DNA and CTAB is not strong enough to form a solid complex as measured by DLS studies in which size distribution and zeta potential are hardly detectable due to the large value of poly-dispersed index. However, when the CTAB concentration is at 50 uM, the zeta potential approaches near zero, indicating that the positive charges of the CTAB molecules neutralize the negatively-charged phosphate backbone of the DNA strands (Table 3.2). The neutralized DNAs aggregate and condense together due to their hydrophobicity, as shown in the largest size distribution by DLS studies (Figure 3.2). The charge neutralization changes the DNA conformation to globular status with the CTAB. It is known that DNA suffers a compaction that goes from an elongated coil state to a compact globule state due to interaction with surfactant molecules [15]. The aggregation of the neutralized DNA facilitates the dye-DNA base and dye-dye interaction, undergoing serious quenching as shown in the emission intensity. This quenching by the aggregation is especially noticeable in ssDNA. It is hypothesized that, even in the compact globule state, dye conjugated on
dsDNA has less chance to contact the quencher, with DNA bases and other dyes, because dsDNA has fewer free bases and is a relatively rigid structure compared to ssDNA (Figure 3.3).

When the CTAB concentration was increased to above 250 uM, the zeta potential became positive, implying that the CTAB forms complete micelle structures with positively charged outer surfaces. In terms of ssDNA, the TAMRA emission intensity recovered to similar values as seen with 0 uM CTAB. The recovered emission correlates to the DLS results of the ssDNA-CTAB complex becoming less than 50 nm size. The negatively charged phosphate backbone in DNA becomes bound to the positively charged micelle surface, resulting in aggregated ssDNA at 50 uM CTAB that is now dispersed with micelles in the

**Table 3.2:** Zeta potential (mV) of complex of CTAB and Dye conjugated DNA

<table>
<thead>
<tr>
<th>[CTAB] (uM)</th>
<th>5</th>
<th>25</th>
<th>50</th>
<th>250</th>
<th>500</th>
<th>2500</th>
<th>5000</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAMRA on ssDNA</td>
<td>NA</td>
<td>-12.5</td>
<td>-0.2</td>
<td>12.5</td>
<td>19.3</td>
<td>24.3</td>
<td>23.2</td>
</tr>
<tr>
<td>TAMRA on dsDNA</td>
<td>NA</td>
<td>-9.8</td>
<td>-3.1</td>
<td>15.3</td>
<td>21.1</td>
<td>26.4</td>
<td>27.7</td>
</tr>
<tr>
<td>TexasRed on ssDNA</td>
<td>NA</td>
<td>-10.3</td>
<td>-0.7</td>
<td>11.3</td>
<td>20.1</td>
<td>24.6</td>
<td>14.2</td>
</tr>
<tr>
<td>TexasRed on dsDNA</td>
<td>-6.4</td>
<td>-11.7</td>
<td>-3.9</td>
<td>18.1</td>
<td>23.6</td>
<td>22.8</td>
<td>26.2</td>
</tr>
</tbody>
</table>
solution and stretched on the micelle surface. Still, the fluorescence emission of TAMRA is being quenched due to free guanine bases in the ssDNA compared to the maximum intensity of TAMRA in dsDNA at 0 uM CTAB. In contrast to the ssDNA-CTAB complex which becomes less than 50 nm size with micelle formation, the size of the dsDNA-CTAB complexes is sustained above 300 nm even with micelle. It is hypothesized that maintaining the large size is based not only on the rigid structure but also the highly negatively charged backbone of the double helix. Even though dsDNA is attached to micelles by electrostatic interaction between the negatively charged backbone and the cationic micelle surface, the opposite side of the backbone also has the potential to interact with another micelle. It is reported that a two-dimensional intercalated hexagonal structure has also been proposed for complexes of DNA with CTAB, arranging DNA strands surrounded by three cylindrical micelles [31, 33]. As the emission intensity was not maximized with a high concentration of CTAB, the aggregation of dsDNA-CTAB complex could still be attributed to the quenching of the TAMRA emission due to dye-dye and dye-DNA base interactions.

3.3.2 TexasRed on DNA

At 0 uM CTAB concentration, when TexasRed conjugated DNA strand (IIa) hybridized with the complementary strand (I), the fluorescence emission increased (Figure 3.1b). However, in contrast to TAMRA emission which became maximized in dsDNA, the TexasRed emission could not be fully recovered even after hybridization, when compared to the maximum intensity of TexasRed ssDNA at 250 uM CTAB. It is hypothesized that the free guanine bases in the complementary strand could be still quenching the TexasRed emission in dsDNA. For ssDNA, the TexasRed emission at 50 uM CTAB was quenched by the aggregation of the neutralized CTAB-DNA complex, which is similar to TAMRA results. However, due to the absence of guanine bases on the DNA strand, it is assumed that the quenching is not as serious as the TAMRA on ssDNA. As the CTAB concentration was
increased to 5000 uM, the TexasRed emission became maximized on ssDNA, implying that the quencher effects from the DNA bases and other dyes were mostly removed by the binding to the micelle surface. It could be assumed that the hydrophobic dyes and linker arms were partially buried in the micelle structures when ssDNA sits on the micelle surface. This might cause the TexasRed dyes to become isolated from the quencher molecules, leading to increased emission. For TexasRed on dsDNA, as the emission is much less sensitive to aggregation with CTAB molecules, the intensity change is different than from TAMRA on dsDNA. It is hypothesized that the difference might be attributed to the different sequences of the conjugated DNA and the material properties of dye such as hydrophobicity and quenching sensitivity by DNA bases. For example, since it is known that the fluorescence emission of TAMRA is more susceptible to be quenched by a guanine base [17, 46], the emission intensity of TAMRA conjugated on strand I, which is already quenched due to DNA bases, could recover the original emission by hybridization and CTAB aggregation.

The emission peak is also depending on DNA strand and micelle formation (3.4). In contrast to dye conjugated on dsDNA, the emission peak shifting of dye conjugated on ssDNA was influenced by the DNA bases and micelle formation. Below CMC (critical micelle concentration), the blue-shifting was clearly observed when DNA becomes double helix which have less free base. If the micelle is present in the solution, it is assumed that the dye conjugated on ssDNA could easily interact with micelle due to flexibility of single strand, causing the blue-shifting of the emission.

### 3.3.3 FRET on DNA

When the TAMRA conjugated DNA strand (Ia) and the TexasRed conjugated strand (IIa) were hybridized without CTAB, FRET transfer between the TAMRA donor and TexasRed acceptor is minimal (Figure 3.5). The inefficient FRET transfer is attributed to the contact quenching between the donor and acceptor dyes, which is supported by the
Figure 3.4: Wavelength change of maximum peak in emission spectrum with respect to CTAB concentration

absorbance spectra where a blue-shifted TAMRA peak and a decreased TexasRed intensity appeared after hybridization. In the case of contact quenching, it is known that the visible absorbance spectrum of the dyes is substantially altered, whereas in the FRET, the visible absorbance spectrum of the dyes is unchanged [34, 42]. However, in 50 uM or higher concentration of CTAB, the absorbance spectrum of the hybridized dyes does not change as it does with pristine dyes. The absorbance results imply that CTAB surrounding the DNA strand can suppress the fluorescence quenching by protecting or sheathing the donor (TAMRA) and acceptor (TexasRed), which facilitates the more efficient FRET. As a result, at 50 uM CTAB concentration where CTAB-dsDNA complexes becomes neutralized, high intensity fluorescence emission from the TexasRed acceptor was observed while excited
Figure 3.5: Fluorescence emission spectra of TAMRA (strand Ia, Black line), TexasRed (strand IIa, Black dot) and hybridized FRET probe (strand Ia+IIa, Red line) with (a) no CTAB and (b) 50 uM of CTAB. (Inset: Absorbance spectra of combining the absorption of the individual strand Ia and IIa (Black), and the hybridized FRET probe (strand Ia+IIa, Red line) in absence (Inset a) and presence (Inset b) of 50 uM of CTAB) (c) Maximum emission intensities of each dye in FRET probe extracted from overall emission spectra in the hybrid (Strand Ia+IIa) as excited at 520 nm
at 520 nm, which is the excitation maximum for the TAMRA donor. This high efficient FRET produced a TexasRed acceptor emission that was almost four fold higher than in the absence of CTAB. This higher FRET is most likely due to reduction of the contact quenching by CTAB, the protection and sheathing of the dye molecules and closer proximity of the donor and acceptor dyes. As explained in the dsDNA experiment, CTAB molecules at 50 uM can surround the phosphate backbone of dsDNA, neutralizing their charges. Since the hydrophobic tail of the surrounding CTAB can sheathe dyes, the contact quenching by DNA bases could be suppressed. Moreover, fluorescence emissions of two dyes conjugated on dsDNA are more easily quenched by dye-dye interaction. In addition, the high density of dye population by aggregation decreases the inter-molecular distance between dye-conjugated DNAs, causing shorter donor-acceptor distances and increasing the emission from TexasRed. The increased emission from the acceptor by aggregation was verified through time-dependent tests (Figure 3.6 ), implying that the emission intensity could be influenced by the size of the CTAB-DNA complex. When 50 uM CTAB was added in the solution, the emission intensity of the acceptor continued to increase during the aggregation in contrast to the decrease in intensity seen by re-dispersion of the aggregation when adding more CTAB.

### 3.4 Conclusion

In this study, the CTAB induced quenching reduction and FRET enhancement mechanisms were investigated for a fluorescent donor dye (TAMRA) and fluorescent acceptor dye (TexasRed) attached at the middle of two 21mer complementary DNA strands. Adding CTAB to the aqueous buffer solution not only changed the solvent polarity, but also the interactions with the DNA, forming a CTAB-DNA complex, which affected the fluorescence emissions of the dyes on the DNA. For CTAB concentrations lower than 50 uM, the solution became less polar and the fluorescence emissions decreased slightly due to the restriction of
Figure 3.6: Time-dependent changes of maximum intensities of TexasRed emission in the FRET probe. Measurement started when corresponding concentration of CTAB is added in the solution, initially. The TexasRed intensities were extracted from overall emission spectra as mentioned in the experimental methods and measurements.
the phenyl ring rotation in the dyes. At 50 uM CTAB, the cation surfactant neutralized the DNA backbone and caused aggregation. For the ssDNA structures, the aggregation enabled fluorescent dye-dye interaction which was attributed to the static quenching at 50 uM CTAB. At CTAB concentrations higher than 50 uM, the quenching disappeared when the ssDNA interacted with micelles causing redispersion of the aggregated DNA. The emission intensities for the hybridized dsDNA were higher than those of ssDNA, due to the rigid structure of the hybridized DNA minimizing the DNA base quenching interactions with the fluorescent dyes. When the TAMRA conjugated DNA strand (Ia) and the TexasRed conjugated strand (IIa) were hybridized without CTAB, contact quenching between the donor and acceptor dyes caused relatively inefficient FRET. In 50 uM CTAB, high efficiency FRET was observed due to CTAB molecules covering or sheathing the fluorescent dyes and suppressing the quenching interactions. Under the best CTAB conditions, the FRET efficiency was almost four fold higher than in the absence of CTAB. In addition, for neutralization of the DNA strands by cationic CTAB aggregates/micelles, FRET may also be enhanced by decreasing the intra-molecular distance between the donor and acceptor dyes. The results also suggest that the improved FRET efficiency between TAMRA donor and TexasRed acceptor are due intra-molecular interaction within the dsDNA, rather than inter-molecular interactions between ds-DNA structures. Overall this study opens the door for using not only CTAB but other surfactant molecules to reduce quenching and improve overall FRET efficiency in DNA structures containing donor and acceptor fluorescent dye molecules. Among other applications, improved FRET efficiency in DNA structures will be valuable for producing DNA based molecular diagnostic assays with much higher sensitivities.

### 3.5 Acknowledgement

Chapter 3 is a reprint of the material as it appears in "CTAB enhancement of FRET in DNA structures, Journal of Biophotonics", by Taeseok Oh, Tsukasa Takahashi, Sejung
Kim, Michael J. Heller, Journal of Biophotonics, 2016. The dissertation/thesis author was the primary investigator and author of this paper.
Chapter 4

Enhanced Fluorescent Resonant Energy Transfer of DNA Conjugates Complexed with Surfactants and Divalent Metal Ions

4.1 Introduction

As reported in the previous chapter 3, when the local concentration of the fluorescent dyes becomes high, for instance, in DNA strands or proteins conjugated with multiple dyes, fluorescent quenching occurs because hydrophobic interactions between the aromatic dye molecules cause them to stack together in order to minimize their contact with water [45, 35]. In order to improve FRET efficiency for DNA diagnostic and other applications we investigated the effect of three different surfactants, Triton X-100, cetyl-trimethylammonium bromide (CTAB), Sodium dodecyl sulfate (SDS) and the divalent cation Mg$^{2+}$ on reducing dimerization and emission quenching in a 21mer oligonucleotide double stranded DNA
(dsDNA) structure, where one strand was conjugated with donor dye (TAMRA) and the other with the acceptor dye TexasRed.

### 4.2 Materials and Methods

The dye-conjugated and non-conjugated 21mer oligonucleotide single strands were prepared as shown in the Table 4.1. The fluorescent donor dye TAMRA (Carboxytetramethylrhodamine) was attached to the thymine base located in the middle of the donor DNA strand (5’-TGT GTG TGT GTT TTT TGG TTT-3’) using a C6-linker (six methylene groups) or a C2-linker (two methylene groups), and the fluorescent acceptor dye TexasRed (sulforodamine) was attached to the adenine base located at the middle of acceptor (complementary) DNA strand (5’-AAA CCA AAA AAC ACA CAC ACA-3’) using a C6-linker (six methylene groups). When hybridized, the TAMRA donor dye and TexasRed acceptor dye should be within about 1 2 nm from each other. The concentrations of the single stranded DNA oligonucleotide solutions were determined by UV-Vis absorbance measurements. For preparing the double stranded (hybridized) FRET constructs, 1 μM of TAMRA conjugated DNA was mixed with 1 μM of TexasRed conjugated complementary strand, then heated to 60°C and cooled down slowly to room temperature for 2 hours. Spectral results such as fluorescent emission and absorbance of the hybridized DNA based FRET systems were obtained by using Tecan spectrophotometer (Infinite M200 Pro, Tecan Inc.). For the fluorescent emission spectra, a 1 μM solution of the hybridized dye-conjugated dsDNA was excited at 520 nm and the emission was measured from 550 nm to 700 nm. In order to examine the dimerization of fluorophores on DNA, the absorbance intensity ratio was calculated by using absorption intensities at 530 nm and 595 nm corresponding to TAMRA dimer peak and TexasRed monomer peak, respectively. All measurements were repeated three times for more reliable result.
Table 4.1: Information on dye conjugated DNA (Chemical structure of the linkers is shown in Fig. S1)

<table>
<thead>
<tr>
<th>Dye</th>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor</td>
<td>C6-linked TAMRA on DNA</td>
<td>5’- TGT GTG TGT G (5-TAMRA)(dT-C2-NH) T TTT TGG TTT-3’</td>
</tr>
<tr>
<td>Donor</td>
<td>C2-linked TAMRA on DNA</td>
<td>5’- TGT GTG TGT G (5-TAMRA)(dT-C6-NH) T TTT TGG TTT-3’</td>
</tr>
<tr>
<td>Acceptor</td>
<td>C6-linked TexasRed on complementary DNA</td>
<td>5’- AAA CCA AAA A (TexasRed-X)(dA-C6-NH) C ACA CAC ACA-3’</td>
</tr>
</tbody>
</table>

4.3 Results

Under ideal conditions, when hybridization of the TAMRA conjugated DNA strand with the TexasRed conjugated DNA strand brings the donor and acceptor dyes into close proximity (about 1-2 nm), the resulting high FRET efficiency is expected to produce high emission of TexasRed acceptor dye. However, compared to the ideal FRET emissions, the fluorescent emissions of the donor dye as well as the acceptor dye were significantly quenched (Figure 4.1). Additionally, the absorbance spectrum intensity at 530 nm increased and intensity at 595 nm decreased when compared to the combination of the original absorbance spectra of two fluorophores. These changes in the emission and absorbance spectra of the dyes are attributed to the static or contact quenching caused by dimerization between TAMRA donor and TexasRed acceptor dyes. It is well-known that dimerization of aromatic structures (fluorescent dyes) often occurs in aqueous solutions, especially if local concentration of the hydrophobic fluorophores is high; for example when fluorophores are conjugated onto single molecules in a fashion that allows them to contact each other [28, 4, 42]. Formation of both homo-dimers and hetero-dimers can cause distinct changes in the absorption spectrum due to coupling of the excited-state energy levels [21]. In terms of H-type aggregates, absorption is allowed only at the highest levels of the excited-state, causing the absorption spectra to be blue-shifted and the fluorescence to be diminished.
Thus, changes in absorbance spectrum, as seen in Figure 4.1 (inset) indicate that the quenched emission is due to the dimerization of two fluorophores, since the absorptions at 530 nm and 595 nm correspond to the TAMRA dimer and TexasRed monomer, respectively [4, 42].

By comparing the linker arm lengths between TAMRA donor dye and backbone of the DNA strand, the shorter C2-linker caused more emission quenching and absorbance shifting than does the longer C6-linker. Due to closer distance between the donor and the acceptor, it is hypothesized that the shorter linker facilitates better dimerization of dyes, causing emission quenching and the lower FRET efficiency. In control studies, where the TAMRA conjugated DNA strand and the TexasRed conjugated DNA strand were hybridized to their complementary DNA strands without dyes, the dimer absorbance peak was not present. This strongly supports that dimerization is occurring when the TAMRA conjugated DNA strand and the TexasRed conjugated DNA strand are hybridized together (Figure 4.2). Thus, it is concluded that the emission quenching after hybridization of donor and acceptor conjugated DNA is mainly attributed to formation of hetero-dimer by contacting the donor to acceptor dyes, not by homo-dimers of the same dyes.

In the first case for reducing quenching and enhancing FRET, Triton X-100, in a concentration range from 1 uM to 10 mM, was added to a solution containing the hybridized TAMRA conjugated DNA strand and the TexasRed conjugated DNA strand. Addition of Triton X-100 alone was observed not to reduce the emission quenching as shown in Figure 4.3 and 4.4. The monomer ratio at 595 nm to 530 nm in absorbance spectra results indicates that the quenched fluorescence emission is still due to the dimerization of the donor and acceptor dyes. Because Triton X-100 is an uncharged polar surfactant, it is likely to have only minimal interaction with negative charged DNA backbone. Even though Triton X-100 forms micelle structures at higher concentrations, critical micelle concentration (CMC) is about 0.2-0.9 mM, it is also assumed that the SDS micelles do not affect the dimerization of
Figure 4.1: (a) Fluorescence emission spectrum excited at 520 nm and (b) Absorbance spectra of TAMRA conjugated ssDNA (black), TexasRed conjugated ssDNA (grey), linearly combined emission spectra of TAMRA conjugated ssDNA and TexasRed conjugated ssDNA (black dash), hybridized C6-linked TAMRA donor and TexasRed acceptor (red) and hybridized C2-linked TAMRA donor and TexasRed acceptor (blue)
**Figure 4.2:** Absorbance spectra of (a) C6-linked TAMRA, (b) C2-linked TAMRA, and (c) TexasRed acceptor conjugating on dsDNA

**Figure 4.3:** Schematics on (a) quenched emission by dimerization without surfactant and (b) enhanced emission by FRET with surfactant in dye-conjugated DNA

dyes conjugated on the ds-DNA.

In the second case where CTAB, a cationic surfactant was added to the solution, the emission and absorption did not change for CTAB concentrations lower than 100 uM. The DNA hybridization still leads to contact quenching between TAMRA donor and TexasRed acceptor producing reduced donor emission and an inefficient FRET. However, by increasing the CTAB concentration to 100 uM and above, the emission of the acceptor now increased, indicating that FRET from the donor to the acceptor becomes favorable and quenching is reduced. The studies from the previous experiments show that the positive charges of the CTAB molecules neutralize the negative charged phosphate backbone of the DNA strands,
Figure 4.4: (a) Emission intensity of TexasRed acceptor and (b) absorbance peak ratio of the TexasRed acceptors monomer to TAMRA donors dimer based on Figure 4.5 and 4.6 with respect to different concentration of surfactants and cations (red: C6-linked TAMRA based DNA FRET system, blue: C2-linked TAMRA based DNA FRET system)
Figure 4.5: Absorbance spectra of C6-linked TAMRA on DNA FRET system with different concentration of (a) Triton X-100, (b) CTAB, (c) SDS, (d) sodium with 10 mM SDS and (e) magnesium with 10 mM SDS causing the DNAs to aggregate and condense together with CTAB molecules due to their hydrophobicity [49]. As absorbance spectrum is shown in Figure 4.5(b) and 4.6(b), the high CTAB concentration causes the increase of the background, which implies that the aggregation formed in the solution. CTAB surrounding the DNA strand can suppress the dimerization and the fluorescence quenching by protecting or sheathing the donor (TAMRA) and acceptor (TexasRed), which facilitates the efficient FRET (Figure 4.7 and 4.8).

In the third case, when SDS, an anionic surfactant, was added to the solution, similar to the experimental results of Triton X-100, the quenched fluorescent emission of dye-conjugated on DNA was not affected. The absorbance ratio of TexasRed monomer to TAMRA dimer demonstrates that SDS does not affect the dimerization of two dyes due to repulsive forces between negatively charged DNA backbone and the anionic SDS molecule. However, for the DNA FRET system with C6-linked TAMRA donor, when sodium cation was added to the solution, the fluorescence emission of the TexasRed acceptor
Figure 4.6: Absorbance spectra of C2-linked TAMRA on DNA FRET system with different concentration of (a) Triton X-100, (b) CTAB, (c) SDS, (d) sodium with 10 mM SDS and (e) magnesium with 10 mM SDS

did increase due to reduced dimerization and the quenching. It is hypothesized that the increased emission by the reduced dimerization is attributed to SDS micelle and cationic sodium ions (Na$^+$) in the solution. Since sodium ions are present in the solution, these cations can form a double layer which screens the repulsive forces between DNA backbone and SDS micelle. Once the SDS micelle forms in the solution and approaches to DNA strand, it could be assumed that the hydrophobic dyes and linker arms are partially introduced into the micelle structures when DNA strand sits on the micelle surface. Inside of the micelle, the donor (with longer C6-linker) and acceptor dyes are prevented from contact quenching leading to increased FRET emission. The hypothesis is supported by additional experiments in which the dimerization was suppressed and the emission was enhanced as the salt concentration increased. When the sodium concentration was increased from 10 mM to 50 mM in 10 mM SDS environment, the fluorescence emission of the acceptor was enhanced about 150%. The further emission enhancement was more clearly observed with divalent
magnesium cations (Mg$^{2+}$), which implies that divalent magnesium cations can neutralize the negative charged DNA backbone more effectively than the monovalent sodium cations. Again, allowing the dsDNA to more closely approach the anionic micelle surface, and the conjugated dyes on DNA to interact with the hydrophobic micelle interior which reduces contact quenching. In contrast to the DNA FRET system with C6-linked TAMRA in which the cations effectively reduces the contact quenching and improves FRET, the monomer ratio and fluorescent emission of the acceptor in the DNA FRET system with C2-linked TAMRA was not influenced by sodium cation. Though monovalent sodium ions were barely effective, the acceptor emission was enhanced and the dimerization was reduced in solutions where the divalent magnesium cation (Mg$^{2+}$) concentration was over 20 mM. Although the repulsive forces between DNA strand and the SDS micelle weaken due to the charge screening effect of sodium cations, it is assumed that the distance between DNA and the SDS micelle is still too far in order for C2 linked TAMRA to introduce the micelle, as compared to the C6 linked TAMRA. Because the divalent magnesium cations can more effectively neutralize the negative charged DNA backbone, this now leads to enhance FRET even with the shorter C2 linker conjugated TAMRA DNA system. Overall, emission quenching was reduced and FRET emissions were significantly enhanced for both DNA systems when using 10 mM SDS and divalent magnesium cations.

### 4.4 Conclusion

Three surfactant/metal ion induced mechanisms for quenching reduction and FRET enhancement were investigated for a hybridized 21mer ds-DNA oligonucleotide construct containing a fluorescent donor (TAMRA) and acceptor (TexasRed) dyes attached at the middle of each strand. When the TAMRA conjugated DNA strand and TexasRed conjugated strand were hybridized without surfactant and divalent metal ions, hydrophobic dimerization interactions between the donor and acceptor caused emission quenching, which leads to
Figure 4.7: (a) Emission spectrum and (b) Absorbance spectrum of TAMRA donor (black bold), TexasRed acceptor on ssDNA (black dash), pristine FRET (gray), FRET with 10 mM of Triton X-100 (yellow), CTAB (blue), SDS (red), SDS with 50 mM NaCl (red dash) and SDS with 50 mM MgCl$_2$ (red dot) in C6 linker based FRET system

Figure 4.8: (a) Emission spectrum and (b) Absorbance spectrum of TAMRA donor (black bold), TexasRed acceptor on ssDNA (black dash), pristine FRET (gray), FRET with 10 mM of Triton X-100 (yellow), CTAB (blue), SDS (red), SDS with 50 mM NaCl (red dash) and SDS with 50 mM MgCl$_2$ (red dot) in C2 linker based FRET system
low FRET efficiency. This is in spite of the fact that both the donor and acceptor dyes on the DNA strands are well within favorable Forster distances ($1/R^6$, about 1.2 nm) for high FRET efficiency. The dimerization and emission quenching was found to be more pronounced in C2-linker TAMRA based FRET system, then in C6-linker TAMRA based FRET system, because the distance between the donor and the acceptor is closer with C2-linker. When Triton X-100, a neutral polar surfactant, was added to the solution at concentrations from 1 uM to 10 mM, no change was observed in emission quenching, indicating no interaction with the DNA strands. However, when CTAB, a cationic surfactant, was added at concentrations of 100 uM or higher the cationic micelles neutralize the DNA backbone inducing aggregation which reduced emission quenching and resulted in higher fluorescent emission (quantum yield) and improved FRET efficiency. This is likely due to cationic CTAB molecules sheathing or insulating the fluorescent dyes and suppressing the quenching due to dimerization and the polar water molecules [54]. In the case of the SDS, an anionic surfactant, the surfactant itself did not reduce emission quenching due to repulsive forces between DNA and SDS micelles. However, when cations such as sodium and magnesium, specifically 50 mM Mg$^{2+}$ where added into 10 mM SDS, both the contact and water molecule quenching were significantly reduced and a very large increase in FRET efficiency was observed. It is hypothesized that the divalent Mg$^{2+}$ cations reduce the repulsive forces on the DNA backbone, allowing interaction of the fluorescent dyes with the SDS micelle. The fluorescent dye-dye contact quenching is broken, but the dyes still are in close proximity for good FRET transfer efficient. Additionally, the sheathing effect shields or insulates the donor and acceptor dyes on DNA excluding the polar water molecules from the structure. The fluorescent dyes now reside in a much less polar environment which further improves fluorescent emission (quantum yield) and overall FRET efficiency. When the C6 linker, connecting TAMRA donor and DNA strand, was replaced with a shorter, C2 linker the dimerization was not reduced as much as it did when using the C6 linker. The longer C6
linker apparently allows penetration of the fluorescent dyes into the hydrophobic micelle regions, reducing the adverse dye-dye interactions. Overall, this study provides important insight for not only using surfactants with cations to reduce quenching and improve FRET efficiency, but also for considering optimal linker structures that connect the fluorescent dye molecules to the DNA strands. With the wide spread use of fluorescent DNA probes and FRET constructs for research and clinical diagnostics, as well other applications, increasing FRET efficiency is of critical importance for continued improvement in their sensitivity, specificity and overall usefulness.

4.5 Acknowledgement

Chapter 4 is a reprint of the material as it appears in "Enhanced Fluorescent Resonant Energy Transfer of DNA Conjugates Complexed with Surfactants and Divalent Metal Ion’s", by Taeseok Oh, Jae-young Choi, Haeun Jang and Michael J. Heller, Analyst, 2016. The dissertation/thesis author was the primary investigator and author of this paper.
Chapter 5

Enhancement of fluorescent resonant energy transfer and the antenna effect in DNA structures with multiple fluorescent dyes

5.1 Introduction

In the nature, a well-known FRET construct is observed in light harvesting complex used by plants and photosynthetic bacteria (Figure 5.1). The complexes consist of proteins and photosynthetic pigments and surround a photosynthetic reaction center to focus energy, attained from photons absorbed by the pigment, toward the reaction center using FRET. A key attribute of light-harvesting systems is the number of donor chromophores that contribute to acceptor emission.

Similar as the the light harvesting complex in photosynthetic system, it should be possible to design DNA structures with a precisely spaced array of donor and acceptor
**Figure 5.1**: Organization of photosystems in the thylakoid membrane: Photosystems are tightly packed in the thylakoid membrane, with several hundred antenna chlorophylls and accessory pigments surrounding a photoreaction center. Absorption of a photon by any of the antenna chlorophylls leads to excitation of the reaction center by exciton transfer (Black arrow). [41]
fluorophores to collect and transfer photonic energy over longer ranges/distances via coupled FRET events. Such DNA structures could act as photonic antennas and wires, which would have many potential applications [8, 40, 18]. Because FRET efficiency among other parameters is determined by a 1/R^6 distance dependency relationship, under ideal conditions the optimal distance for positioning donor to donor and donor to acceptor fluorescent groups on a DNA strand is from about 0.34 nm to 1.4 nm or a one to four base pair separation. From 1.4 nm to 2.4 nm or four to seven base pair separation the FRET efficiency is intermediate to low, while beyond 2.4 nm or seven base pair spacing the FRET efficiency drops off dramatically. Thus, in DNA structures with multiple fluorescent donor groups designed for long range FRET, the donor to donor dye resonant energy transfer efficiency must be intermediate to high inorder for the energy that was collected by the more distant donors (beyond 2.4 nm) to reach the fluorescent acceptor dye. However, when multiple fluorophores are in close proximity and can contact each other the resulting hydrophobic stacking interactions leads to the formation of donor/donor and donor/acceptor dimers. Such contact or dimer formation between the dyes is one of several quenching mechanisms that can significantly reduce the fluorescent emissions and overall FRET efficiencies in DNA structures designed for long range transfer and antenna properties [62, 44].

Quenching of fluorophores on the DNA structures can also occur by contact with water or other polar molecules, as well as with DNA bases where electron-transfer from the base to the excited singlet state of a dye can sometime quench the fluorescence emission [6, 52, 61]. Such DNA base quenching is mostly due to guanine, which has the lowest oxidation potential among the four DNA bases [17, 47]. As was reported in earlier studies (Chapter 3 and 4), the interaction and association of fluorescent dye conjugated ds-DNA structures with certain surfactant micelles and metal ions was found to significantly reduce dimerization and emission quenching and improve short range FRET efficiencies, where the donor to acceptor dye distance was less than 1.7 nm or five base pairs.
In this study, we now investigate the effect of surfactants and metal cations on reducing quenching and enhancing the long range FRET efficiency and antenna effects in 21mer ds-DNA structures with different arrangements of three TAMRA donor dyes and a single TexasRed acceptor dye. In these DNA structures, efficient donor to donor FRET is required as the distance of the distal donor dyes to the acceptor dye becomes greater than 1.7 or five base pairs, which is beyond the optimal FRET distance.

5.2 Materials and Methods

Both dye-conjugated 21mer single-stranded (ss) oligonucleotides and 21mer un-conjugated single-stranded (ss) oligonucleotides were supplied by Trilink Inc, San Diego, CA. For the donor 21mer ss-oligonucleotides, three fluorescent TAMRA dyes (CarboxytetramethylRhodamine) were attached to the thymine bases via C6-linkers (six methylene groups) with a 3 base spacing (5- TGT GTG (dT-C6-NH-5-TAMRA) GTG (dT-C6-NH-5-TAMRA) TTT (dT-C6-NH-5-TAMRA) TGG TTT-3) and a 7 base spacing (5- TG (dT-C6-NH-5-TAMRA) GTG TGT G (dT-C6-NH-5-TAMRA) T TTT TGG (dT-C6-NH-5-TAMRA) TT-3). For the acceptor 21mer ss-oligonucleotides a single fluorescent TexasRed (sulforodamine) dye was attached via C6-linkers (six methylene groups) to the 10th adenine base from the 5-terminal position (5- AAA CCA AAA A (dA-C6-NH-TexasRed-X) C ACA CAC ACA-3), and to the 3rd adenine base located from the 5-terminal position (5- AA (dA-C6-NH-TexasRed-X) CCA AAA AAC ACA CAC ACA-3). A control complementary 21mer oligonucleotide without a TexasRed dye was also synthesized. Table 5.1 shows the oligonucleotide sequences and positions of the donor and acceptor dyes and Figure 5.2 shows simple schematic representations of the ss-DNA and ds-DNA structures. The concentrations of the ss-DNA oligonucleotide solutions were determined by UV-Vis absorbance measurements. For preparing the hybridized double-stranded (ds) DNA structures, 1 µM of the three TAMRA conjugated DNA (Donor sequence I and II) was mixed with 1 µM of
the TexasRed conjugated complementary strand (Accepter sequence III and IV) or with the blank complementary strand (No acceptor sequence V) in 0.5X PBS, then heated to 60 °C and cooled down slowly to room temperature 20 °C for 2 hours. Fluorescent emission and absorbance spectra of the hybridized ds-DNA FRET structures were obtained using a multi-mode Microplate reader (Infinite M200 Pro, Tecan Inc). The excitation and emission used for the TAMRA donor dyes were (ex. max 555 nm) and (em. max 580 nm) and the TexasRed dye was (ex. max 595 nm) and (em. max 615 nm) respectively.

The FRET efficiency of the donor-acceptor DNA hybrids was measured experimentally in several ways. Basically, quantification methods for FRET efficiency requires monitoring the change of emission intensity or quantum yield of the acceptor fluorophore with respect to presence and absence of the donor fluorophore [40]. However, in the multi-donor and single acceptor DNA systems where the emission intensity of the donors are highly influenced (quenched) by dimerization, the lower FRET efficiencies are more difficult to quantify exactly. However, they do set a reliable lower limit for the other measurements. The long distance FRET transfer and antenna effect measurements are an effective way to characterize the overall light-harvesting or collection capability of the multi-donor single acceptor DNA structures. In order to quantify the FRET performance for the three TAMRA donors and one Texas Red acceptor ds-DNA structures under each of the environmental conditions (with and without surfactants and metal ions), the Antenna Effect Value was determined by measuring the intensity ratio of TexasRed acceptor emission when multiple TAMRA donors are excited at 565 nm, to the TexasRed acceptor emission when the acceptor alone is excited at 595 nm, as shown in equation 5.1.

\[
\text{Antenna effect} = \frac{\text{Acceptor Emission}_{\text{Multiple Donors Excited}}}{\text{Acceptor Emission}_{\text{Single Acceptor Excited}}} \quad (5.1)
\]

In this study all measurements were repeated three times for more reliable results. Generally, the distance between donor to donor and donor to acceptor groups is referred
to as the Forster distance. Among other parameters, FRET efficiency has a $1/R^6$ distance dependency with the Forster distance. Under ideal or theoretical conditions the FRET efficiencies for a single fluorescent donor and single fluorescent acceptor conjugated to a ds-DNA structure would be High (100 % to 90 %) for Forster distances from about 0.34nm to 1.4 nm (1-4 base pair separation); Intermediate (80 % to 40%) for Forster distances from 1.4nm to 2.4 nm (4 to 7 base pair separation); and Low (≤ 30%) for distances greater than 2.8nm or 8 base pair separation, where the FRET efficiency then drops off rapidly to 0% because of the $1/R^6$ distance dependency. For a single donor and single acceptor system at the closest Forster distance and under ideal conditions the maximum FRET efficiency cannot be greater than 100%, nor can it have a value greater than 1 for the emission acceptor-donor excited / emission acceptor-acceptor excited ratio. However, in cases where there are multiple donors including donors beyond the Forster distance that have efficient donor to donor FRET that produce an antenna effect, then the overall FRET efficiency measured for a system with multiple donors and a single acceptor group can be greater than 100%, and it can have a value greater than 1 for the acceptor emission-multiple donors excited / emission acceptor-single acceptor excited ratio (Antenna Effect Value). By way of an example, an Antenna Effect Value of 1.5 would mean that the ds-DNA structure with multiple TAMRA donors excited at 555 nm produced a 150% increase in the TexasRed acceptor emission at 610 nm, compared to the maximum emission value at 610 nm for the single TexasRed acceptor when excited at 595 nm.

5.3 Results

In order for better interpretation of the experimental results, Table 5.1 shows the actual base sequences and positions of the fluorescent TAMRA donor dyes and the fluorescent Texas Red acceptor dye in the ss-DNA and dsDNA structures, while Figure 5.2 shows the schematic representations of ss-DNA sequences and the hybridized FRET ds-DNA
Figure 5.2: Schematics representation for: (a) the three fluorescent TAMRA donor dyes on ss-DNA sequences and in dsDNA hybrid structures without the presence of the fluorescent TexasRed acceptor dye; and (b) the three fluorescent TAMRA donors in FRET ds-DNA hybrid structures with the fluorescent TexasRed acceptor dye present in the complementary sequence.
Table 5.1: DNA sequences and the positions of donor and acceptor dyes

<table>
<thead>
<tr>
<th>DNA Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor sequence I</td>
<td>5’- TGT GTG (5-TAMRA)(dT-C6-NH) GTG (5-TAMRA)(dT-C6-NH) TTT (5-TAMRA)(dT-C6-NH) TTT (5-TAMRA)(dT-C6-NH) TGG TTT-3’</td>
</tr>
<tr>
<td>Donor sequence II</td>
<td>5’- TG (5-TAMRA)(dT-C6-NH) GTG TGT G (5-TAMRA)(dT-C6-NH) T TTT TGG (5-TAMRA)(dT-C6-NH) TT-3’</td>
</tr>
<tr>
<td>Acceptor sequence III</td>
<td>5’- AAA CCA AAA A (TexasRed-X)(dA-C6-NH) C ACA CAC ACA-3’</td>
</tr>
<tr>
<td>Acceptor sequence IIII</td>
<td>5’- AA (TexasRed-X)(dA-C6-NH) CCA AAA AAC ACA CAC ACA-3’</td>
</tr>
<tr>
<td>No Acceptor sequence IV</td>
<td>5’- AAA CCA AAA AAC ACA CAC ACA-3’</td>
</tr>
</tbody>
</table>

structures. The Control A and B three TAMRA dye ss-DNA sequences and Control C and D hybridized three TAMRA dye ds-DNA structures without the presence of the TexasRed dye in the complementary strand are shown in Figure 5.2a, and the FRET E, F, G and H three TAMRA donor dye hybridized ds-DNA structures with the TexasRed acceptor dye present in the complementary sequence are shown in Figure ??b. In this study the results for the Control ssDNA, Control ds-DNA hybrid structures, and FRET ds-DNA hybrid structures without surfactants or metal ions are designated as ”Pristine”. With regard to the FRET dsDNA hybrid structures E, F, G and H, the dsDNA hybrid structure G represents the best test case for demonstrating long range FRET transfer and the antenna effect.

5.3.1 Three TAMRA dye conjugated DNA structures without Texas-Red acceptor

Before carrying out FRET experiments on the ds-DNA structures with multiple TAMRA donor groups and a single TexasRed acceptor group in the complementary oligonucleotide strand, the fluorescence and UV absorbance properties of the three TAMRA donor ss-DNA (Control sequence A and B) and the ds-DNA (Control hybrid C and D) with the
3 base and 7 base spacing and no TexasRed acceptor present in the complementary DNA strand, were tested without surfactants or metal ions.

As shown in Figure 5.3a, without the addition of surfactants and metal ions, lower fluorescent emission intensities were observed for the TAMRA dyes in the ss-DNA sequences A, B and the hybridized ds-DNA sequence C indicating significant quenching of the dyes, while a higher emission intensity was observed for the hybridized ds-DNA sequence D indicating much less quenching occurred in this structure. This reduced quenching in the ds-DNA sequence is D may be attributed to the 7 base spacing of the TAMRA dyes together with the more rigid double-stranded hybrid structure not allowing any contact to occur between the dyes. Additionally, the absorbance spectrum intensities for the TAMRA dyes in the ss-DNA sequence A, B and the ds-DNA structure C, increased at 524 nm and decreased at 556 nm when comparing to absorbance spectrum of TAMRA dyes in ds-DNA D (Figure 5.3b). The changes in emission and absorbance spectra are mostly attributed to the static quenching by dimerization of TAMRA fluorophores. As has been reported in previous studies (Chapter 3 and 4), contact of fluorescent dyes in aqueous solutions often leads to dimerization, especially if the local concentration of hydrophobic fluorophores is high or they are in close proximity; for example, when they are conjugated in close proximity on a DNA molecule. It is also well-known that formation of homo- and heterodimers can cause distinct changes in the absorption spectrum due to coupling of the excited-state energy levels. In terms of H-type aggregates, absorption is allowed only to the top area of the excited-state, absorption is blue-shifted and the fluorescence emission intensity is diminished [42, 48]. Thus, changes in the absorbance spectrum confirm that the emission quenching is induced by the dimerization of the fluorophore dyes, where the absorptions at 556 nm 524 nm correspond specifically to the TAMRA monomer and dimer, respectively. As seen in Figure 5.3b, lower 556 nm monomer to 524 nm dimer ratios were observed for the TAMRA dyes in the ss-DNA sequences A and B and the hybridized ds-DNA sequence
C, indicating significant dimerization of the dyes, while a higher ratio was observed for the hybridized ds-DNA sequence D indicating much less dimerization. Significant dimerization would be expected in ss-DNA sequence A and ds-DNA C with the shorter 3 base spacing of the TAMRA groups, as well as in the ss-DNA sequence C which even though it has 7 base spacing of the TAMRA groups, the single stranded DNA structure is flexible enough to allow the TAMRA groups to make contact. However, the ds-DNA sequence D with 7 base spacing is rigid enough to prevent any TAMRA dye contact from occurring.

When Triton X-100 was added to the solution, the emission quenching and dimerization was still observed for the three TAMRA dyes in ss-DNA sequences A and B, and the ds-DNA sequence C (Figure 5.3a and 5.3b). Because Triton X-100 is un-charged (neutral), the surfactant has little interaction with negative charged DNA backbone and does not appear to affect the dimerization of the TAMRA dyes. However, the emission intensities of the TAMRA dyes in the ds-DNA structure D decreased as the Triton X-100 concentration increased due to the less polar environment (Chapter 3). It is well known that Rhodamine type dyes such as TAMRA are highly fluorescent in polar solvents and the high quantum yield is maintained by restricting the rotation of the phenyl rings in Rhodamine dyes by a bridging oxygen atom (Chapter 4). Thus, as the Triton X-100 cause the aqueous solution to be less polar the emission intensity of TAMRA monomers in the hybrid D decreases.

When CTAB, a cationic surfactant, was added to the solutions, the emission and absorption did not change until a concentration of 100 µM CTAB was reached. At 100 µM CTAB the absorbance spectrum intensity increases, which implies that aggregation has formed in the solution (Figure 5.4a and 5.4b). The positively charged CTAB molecules neutralize the negative charged phosphate backbone of the DNA strands, causing the DNA structures to aggregate and condense together with CTAB molecules due to their hydrophobicity [42].

In this neutralized and aggregated state the fluorescence and absorbance properties
Figure 5.3: Fluorescent and UV absorption spectral analysis results for the three fluorescent TAMRA donor dye ss-DNA sequences (A and B) and ds-DNA hybrid structures (C and D) without the Texas Red acceptor dye present: (a) maximum fluorescence emission intensities for the three fluorescent TAMRA donor dye ss-DNA structures with and without surfactants and (b) the monomer absorbance spectra ratios (A556nm/A524nm) for the control sequences A (blank gray) and B (blank black), and the control ds-DNA hybrids C (filled gray) and D (filled black)
of TAMRA dyes on the DNA are influenced not only by dimerization, but also by other quencher molecules such as the guanine bases. For instance, while emission quenching occurred as the monomer ratio was decreased in the control hybrid D, the emission barely changed as the monomer ratio increased in the other control hybrids A, B and C (Figure 5.3a and 5.3b). Thus, it is assumed that the emission is influenced by not only the dimerization but also other quenchers such as guanine bases in DNA. However, when CTAB concentration was increased above 100 µM, the emission and the monomer ratios became similar to the ratio for the pristine structures (Figure 5.3b). Since CTAB molecules form micelle structures at these higher concentrations, the negatively charged phosphate backbone in DNA becomes bound to the positively charged micelle surface, resulting in aggregated DNA structures dispersed on the CTAB micelles in the solution. As a result of re-dispersion of the aggregated DNA structures in the solution, the three TAMRA dye conjugated DNA structures become similar to the original configurations, and show spectral properties like the pristine structures.

In the third case, when SDS, an anionic surfactant, was added to the solutions, results were similar to what was observed for Triton X-100. The fluorescent emission and dimerization of TAMRA dye conjugated DNA structures (control A, B and C) were not affected due to repulsive forces between negatively charged DNA backbone and the anionic SDS molecules. However, when cations such as sodium (Na\(^+\)) and magnesium (Mg\(^{2+}\)) were added to the solution, the fluorescence emission intensity of TAMRA dye conjugated DNA increased due to the reduction of dimerization. Apparently, the addition of cations forms a double layer which screens the repulsive forces between DNA backbone and SDS micelle, which now allows the TAMRA dyes to have hydrophobic interaction with interior of the micelle structures. As the TAMRA dyes becomes isolated in the micelle structure, dimerization is reduced and fluorescent emission is increased. This emission enhancement was more clearly observed with divalent magnesium cation (Mg\(^{2+}\)) than with monovalent
Figure 5.4: Absorbance spectra of (a) 3 base spaced three TAMRA on ssDNA, (b) 3 base spaced three TAMRA on dsDNA, (c) 7 base spaced three TAMRA on ssDNA and (d) 7 base spaced three TAMRA on dsDNA with respect to surfactant and cation conditions.
sodium cation (Na\(^+\)), which is most certainly due to more effective reduction in the DNA backbone negative charge by the Mg\(^{2+}\) cations. With regard to the TAMRA conjugated hybrid D, the decreasing solvent polarity resulting from increasing SDS concentration causes a reduction in emission intensity, which is similar to the results observed for Triton X-100.

### 5.3.2 Enhanced FRET and Antenna Effect in dsDNA Structures

In the initial case without surfactants or metal ions, the three TAMRA dye conjugated DNA strands (I, II) when hybridized to the TexasRed conjugated strands (III, IV), the antenna effect values for the FRET dsDNA structures E, F, G, and H were only 1.39, 1.54, 0.61 and 1.42, respectively (Figure 5.5a and 5.5b). These relatively low values were observed in spite of the fact that the TAMRA donor to donor dyes (E and G) and TAMRA donor to TexasRed acceptor dye (E, F and H) on the FRET dsDNA hybrid structures were well within favorable Forster distances (1/\(R^6\), about 1-2 nm), which should theoretically have high FRET efficiency. As was shown in the previous section, hydrophobic interactions between donor dyes (fluorophores) in close proximity causes dimerization and quenching that significantly reduces the fluorescent emission intensity. It certainly likely that FRET dsDNA hybrids E and G have more severe dimerization, which would account for the low antenna effect value inefficient FRET. This appears especially true for the FRET ds-DNA hybrid G, with the lowest antenna effect 0.6, where all three TAMRA donors and the Texas Red acceptor are in close proximity (donors with 3 base spacing and donor to acceptor with equivalent of 1-2 base spacing).

In the first case for using a surfactant for reducing dimerization and enhancing FRET in the dsDNA hybrids E, F, G and H, the non-ionic surfactant Triton X-100 was tested at concentrations that ranged from 1 \(\mu\)M to 10 mM. As shown in figure 5.5a and 5.5b, the addition of Triton X alone did not enhance FRET or the antenna effect. Because Triton X-100 is an uncharged non-ionic polar surfactant, it is likely to have only minimal interaction
Figure 5.5: Antenna effect values for FRET dsDNA hybrid structures. (a) Antenna effect values for FRET ds-DNA hybrid structures E (Blue), F (Red), G (Green), and H (Yellow) with and without surfactants and metal ions; and (b) plot of the maximum FRET values for dsDNA hybrid structures E (Blue), F (Red), G (Green), and H (Yellow) in each surfactant/metal ion environment.
Figure 5.6: Maximum intensity of TAMRA donors at 580nm (blank bars) and Texas Red acceptor at 610nm (color bars) by decomposition (removal TAMRA emission overlap from Texas Red emission) for FRET hybrid E (Blue), F (Red), G (Green), and H (Yellow) when excited at 555nm (Inset: maximum fluorescent emission intensity for Texas red acceptor under each surfactant/metal cation condition)
with negative charged DNA backbone, as was discussed in the previous section.

In the second case, where the cationic surfactant CTAB was used at concentrations of 100 µM and above, both the antenna effect value and fluorescent dye emissions increased, especially in FRET dsDNA hybrid structures E and G (Figure 5.5a and 5.5b and Figure 5.6). However, the donor and acceptor intensity for the emission spectrum barely increased compared to the acceptor intensity for FRET dsDNA hybrids in the SDS and cation environment (Figure 5.5a and 5.5b). It is hypothesized that DNA neutralization and aggregation in solution with 100 µM or higher concentrations of CTAB causes not only a decrease of dimerization between the donor fluorophores through interaction with the micelles, but also further reduces emission quenching produced by other quenchers such as the guanine bases.

In the third case, FRET dsDNA hybrid structures in solutions with SDS concentrations ranging from 1µM to 1mM did not show any significant increase in their antenna effect values (Figure 5.5a and 5.5b). This is a strong indication that donor and acceptor dye dimerization was still occurring. However, when sodium (Na⁺) or magnesium (Mg²⁺) cations were also present in the SDS solutions, the antenna effect values were significantly increased (Figure 5.5a and 5.5b), as were the fluorescent dye emission intensities (Figure 5.6). These increases were most pronounced for the FRET dsDNA hybrid E and G structures, where the dimerization of the three TAMRA donor dyes in closer proximity (3 base spacing) was significantly reduced. As discussed in the previous section, it is hypothesized that screening effect of negatively charged DNA backbone by the cations (Mg²⁺ in particular) allows the hydrophobic TAMRA dye fluorophores to form hydrophobic interaction with the SDS micelles that greatly reduces the dye dimerization and quenching, resulting in improved fluorescent dye emissions and quantum yield (TexasRed). In the best results case for reduced dimerization using SDS and Mg²⁺ cations, the overall antenna effect values where increased from 1.39 to 2.16 for E, 1.54 to 1.84 for F, 0.61 to 1.80 for G and 1.42 to 1.61 for H (see Figure 5.5b). This is about a 15 % increase for the F and H dsDNA
hybrid structures which had three TAMRA donors with 7 base spacing, where the Texas Red acceptor on the complementary strand was directly across from the center TAMRA in the F structure (equivalent to a 1-2 base spacing), and directly across from the 3-terminal position TAMRA in the H structure (equivalent to a 1-2 base spacing). The antenna effect values increased more than 50% for the E dsDNA structure with three TAMRA donors at 3 base spacing and the Texas Red acceptor directly across from the center TAMRA donor (equivalent to a 1-2 base spacing). Most interestingly, there was more than a 100% increase for the G dsDNA structure with three TAMRA donors at 3 base spacing and the Texas Red acceptor at a 3 base spacing across from the 3-terminal TAMRA donor. In addition to the FRET ds-DNA structure G having benefited from the reduced dimerization and quenching effects by SDS surfactant and Mg\(^{2+}\) ion interactions, the relatively high final Antenna Effect Value of 1.80 provides the most convincing evidence for extended FRET and the antenna effect. In the G dsDNA structure, the two most distal TAMRA donors with a 7 base and 11 base spacing are beyond the Forster distance for efficient photonic transfer directly to the Texas Red acceptor.

Finally, the best case FRET spectral information obtained in this study was also analyzed and presented in an alternate fashion. Figure 5.7 again shows the fluorescence emission spectrum data for the Texas Red acceptor in FRET dsDNA hybrids E, F, G, and H with and without 10mM SDS with 50mM Mg\(^{2+}\) cation. The fluorescence emission spectrum of the Texas Red acceptor at 610nm with excitation at 555nm (ex max TAMRA) without SDS and Mg\(^{2+}\) cation is shown as the dashed line (—), the fluorescence emission spectrum of the Texas Red acceptor at 610nm with excitation at 595nm (ex max Texas Red) with 10mM SDS and 50mM Mg\(^{2+}\) ion is shown as the gray line, and the fluorescence emission spectrum of the Texas Red acceptor at 610nm with excitation at 555nm (ex max TAMRA) with 10mM SDS and 50mM Mg\(^{2+}\) cation is shown as the solid color line. The results presented in this fashion again show the difference between the E and G dsDNA structures.
and the F and H the dsDNA structures, and overall help collaborate this study's conclusions.

5.4 Conclusion

Three surfactants and metal cation induced mechanisms for fluorescent quenching reduction and FRET and antenna enhancement were investigated using ss-DNA and hybridized dsDNA 21mer oligonucleotide constructs containing three fluorescent TAMRA donor dyes and one fluorescent TexasRed acceptor dye on a complementary 21mer oligonucleotide (see Table 5.1 and Figure 5.2). For the three TAMRA ssDNA and dsDNA structures with close inter-TAMRA distance (3 base spacing) hydrophobic based dimerizing interactions between the donor fluorophores lead to quenching and low fluorescence emissions. When Triton X-100, a neutral polar surfactant, was added to the solution at concentrations from 1 µM to 10 mM, the dimerization and the emission quenching of TAMRAs did not change, indicating no interaction with the DNA strands. However, when 100 µM of CTAB was added in the solution, the cationic surfactant neutralizes the DNA backbone inducing aggregation which affects random behavior of the emission and dimerization of TAMRAs. In this aggregated form, it is assumed that the fluorescent emission is influenced by not only the dimerization but also other quenchers such as guanine bases in DNA. In the case of the SDS, an anionic surfactant, the surfactant itself did not reduce emission quenching due to repulsive forces between DNA and SDS micelles. However, if concentration of cations such as sodium and magnesium, are increased in 10 mM SDS solution, the dimerization of TAMRA dyes was significantly reduced and a very large increase in fluorescence emission was observed, especially in highly dimerized TAMRAs (strand IA and IB). It is hypothesized that the cations reduce the repulsive forces between the DNA backbone and the SDS micelle, allowing interaction and introduction of the fluorescent dyes into the SDS micelle by hydrophobic attraction. The sheathing effect shields and/or insulates the TAMRA dyes conjugated on DNA, reducing dimerization and enhancing the fluorescent emission.
Figure 5.7: Fluorescence emission spectrum of the Texas Red acceptor in FRET dsDNA hybrids (E, F, G, H) with and without 10mM SDS with 50mM Mg$^{2+}$ ion. Fluorescence emission spectrum of the Texas Red acceptor at 610nm with excitation at 555nm (ex max TAMRA) without SDS and Mg$^{2+}$ ion (dash line —-). Fluorescence emission spectrum of the Texas Red acceptor at 610nm with excitation at 595nm (ex max Texas Red) with 10mM SDS and 50mM Mg$^{2+}$ ion (gray line). Fluorescence emission spectrum of the Texas Red acceptor at 610nm with excitation at 555nm (ex max TAMRA) with 10mM SDS with 50mM Mg$^{2+}$ ion (solid color line)
When the three TAMRA donor sequences (I and II) and the TexasRed acceptor sequences (III and IV) were hybridized to form the FRET dsDNA structures E, F, G and H, it was observed for all cases without the addition of surfactant and cations that dimerization of the fluorophores caused emission quenching leading to relatively low FRET efficiency and antenna effect values. Although non-ionic Triton X-100 did not influence the acceptor emission or the antenna effect, the antenna effect values did increase for the cationic CTAB surfactant at 100 µM and higher concentrations. However, the emission intensity of the TAMRA donor and TexasRed acceptor barely increased, implying that the CTAB surfactant might not best suited to enhance FRET and the antenna effect in dsDNA hybrid structures due to heavy aggregation induced by DNA neutralization.

While the anionic SDS surfactant did not interact with the FRET dsDNA hybrid structures due to repulsive electrostatic forces, the antenna effect values did increase significantly when divalent magnesium (Mg\(^{2+}\)) cation was also present in the 10 mM SDS solution. Apparently, as SDS micelles could now approach the dsDNA structures due to the charge screening effect of Mg\(^{2+}\) cations, the hydrophobic fluorophores were able to interact with the interior of the micelle, which reduces the dimerization and leads to an overall enhancement of fluorescent dye emissions, quantum yields and the antenna effect values (2.16 for E, 1.84 for F, 1.80 for G and 1.61 for H). With regard to the FRET dsDNA hybrid structure G, which represents the best case for discriminating true long range FRET and antenna effects, the high Antenna Effect Value of 1.80 provides convincing evidence that this was occurring. In the G dsDNA structure, the two most distal TAMRA donors with a 7 base and 11 base spacing are clearly beyond the Forster distance for transfer of photonic energy directly to the Texas Red acceptor. In order to account for the high Antenna Effect Value of 1.80, the three TAMRA donors must be FRET coupled as an antenna that allows effective transfer of photonic energy captured by all three donors to be transferred by the proximal TAMRA donor (3 base spacing) to the TexasRed acceptor.
Overall, this research study provides important insights and methods for not only using surfactants with cations to reduce fluorescent dye dimerization and quenching, but also for considering more optimal positioning of multiple fluorescent donor dye and acceptor dye molecules in unique dsDNA structures designed with long range FRET and antenna properties. Such novel FRET dsDNA structures would be useful for enabling a variety of more sensitive detection systems for research and clinical diagnostic applications, as well as for creating new programmable self-assembling 2D and 3D DNA based photonic nanostructures.

5.5 Acknowledgement

Chapter 5 contains material that has been submitted for publication as "Enhancement of fluorescent resonant energy transfer and the antenna effect in DNA structures with multiple fluorescent dyes", by Taeseok Oh, Sejung Kim, Jae-young Choi, Haeun Chang and Michael J. Heller, Small (submitted), 2016. The dissertation/thesis author is the primary investigator and author of this paper.
Chapter 6

Summary and Future work

6.1 Thesis summary

6.1.1 CTAB enhancement of FRET in DNA structures

The effect of cetyl-trimethylammonium bromide (CTAB) on enhancing the fluorescence resonance energy transfer (FRET) between two dye-conjugated DNA strands was studied using fluorescence emission spectroscopy and dynamic light scattering (DLS). For hybridized DNA where one strand is conjugated with a TAMRA donor and the other with a TexasRed acceptor, increasing the concentration of CTAB changes the fluorescence emission properties and improves the FRET transfer efficiency through changes in the polarity of the solvent, neutralization of the DNA backbone and micelle formation. For the DNA FRET system without CTAB, the DNA hybridization leads to contact quenching between TAMRA donor and TexasRed acceptor producing reduced donor emission and only a small increase in acceptor emission. At 50 uM CTAB, however, the sheathing and neutralization of the dye-conjugated dsDNA structure significantly reduces quenching by DNA bases and dye interactions, producing a large increase in FRET efficiency, which is almost four fold higher than without CTAB.
6.1.2 Enhanced Fluorescent Resonant Energy Transfer of DNA Conjugates Complexed with Surfactants and Divalent Metal Ions

Dimerization and resultant quenching of donor and acceptor dyes conjugated on DNA causes loss of fluorescent resonant energy transfer (FRET) efficiency. However, when complexed with surfactants and divalent metal ions, sheathing effects insulate and shield the DNA structures, reducing dimerization and quenching which leads to significant enhancement of FRET efficiency.

6.1.3 Enhancement of fluorescent resonant energy transfer and antenna effect in multiple fluorescent dye DNA structures using surfactants and metal ions

This study investigates approaches for enhancing long range fluorescent energy transfer (FRET) in order to produce an antenna effect in DNA structures with multiple fluorescent donors and a single fluorescent acceptor group using surfactants and metal ions to lower emission quenching due to the stacking and dimerization of the fluorescent dyes. Double-stranded (ds) DNA structures were formed by hybridization of 21mer DNA oligonucleotides with different arrangements of three fluorescent TAMRA donor dyes to a complementary 21mer oligonucleotide with one fluorescent TexasRed acceptor dye. The surfactants and metal ions tested for their ability to reduce emission quenching due to fluorescent dye dimerization and other mechanisms included Triton X-100, cetyltrimethyl ammonium bromide (CTAB), and sodium dodecyl sulfate (SDS), along with sodium ions (Na+) and divalent magnesium ions (Mg++). It was first observed in ds-DNA structures with three TAMRA dyes that strong hydrophobic stacking interactions and dimerization between the dyes caused emission quenching and absorbance shifting even when the TexasRed acceptor dye was not present in the complementary DNA strand. Also, when the inter
TAMRA distances were closer (3 base pair spacing), dimerization and emission quenching were found to be even more pronounced. When the neutral (uncharged) surfactant Triton X-100 was added to the ds-DNA with three TAMRA donors and one TexasRed acceptor, dye dimerization and emission quenching remained unaffected. However, for the positively charged CTAB surfactant at concentrations of 100 uM and above, the neutralization of the negatively charged ds-DNA backbone by interaction with the cationic surfactant micelles was found to reduce dimerization and emission quenching, which now resulted in much higher FRET efficiency and an enhanced antenna effect. This improvement is likely due to the CTAB molecules actually covering or sheathing the fluorescent donor and acceptor dyes and breaking up the dimerized complexes. While the negatively charged SDS surfactant alone was not able to reduce dimerization and emission quenching due to repulsive forces between DNA and SDS micelles, the addition of cations such as sodium ions (Na+) and divalent magnesium ions (Mg++) did lead to a reduction in the dimerization and emission quenching resulting in much higher FRET efficiency and an enhanced antenna effect. It appears that when the repulsive electrostatic forces are screened by the cations, the SDS micelles can approach the ds-DNA structure thereby sheathing or insulating the TAMRA and TexasRed dyes. Similar results were observed for ds-DNA structures with three TAMRA donor dyes and without the TexasRed acceptor dye, where the TAMRA emissions were also higher in the presence of SDS and cations. In the absence of surfactants, donor-donor dye dimerization and heterodimer donor-acceptor dye dimerization caused emission quenching and a lower FRET efficiency and reduced antenna effect in all the ds-DNA structures. Overall, this study provides a strategy for using certain surfactants and cations to reduce fluorescent dye quenching and improve the overall long distance FRET efficiency and the antenna effect in DNA structures with multi-donor and single acceptor fluorescent dye groups.
6.2 Further Experiments and Future work

Additional experiments have been performed in order to enhance the antenna effect and realize the long-range DNA photonic wire. As shown in the Figure 6.2, five TAMRA donors and one TexasRed acceptor conjugated 35 base pair dsDNA were tested.

Although serious emission quenching occurred due to dimerization of six dyes conjugated on dsDNA, it was expected to show enhancing FRET and high antenna effect value when adding SDS micelle and magnesium cation could reduce the dimerization. As similar results shown in the chapter 5, SDS micelle and Mg cations enables for dyes not to form the dimerization, which attributes that fluorescent emission from acceptor increased. The antenna effect of the hybrid X and Y increased from 0.68 to 2.36 and from 0.89 to 2.53, respectively. It is concluded that the antenna effect does not enhance above to 3.0 even though donor ratio of the acceptor becomes five and the dimerization could be suppressed by SDS micelle and Mg cations. It is assumed that quantum yield of acceptor fluorophores
Figure 6.2: Schematics of five TAMRA donors and one TexasRed acceptor on ds-DNA structures with/without SDS micelle and cations which relates to maximum values of amplified emission should be considered in order to figure out mechanism of multi-donor FRET system in details.
Bibliography


[38] I. Medintz and N. Hildebrandt. *FRET-Förster Resonance Energy Transfer: From Theory to Applications*. John Wiley & Sons, 2013.


