

UNIVERSITY OF CALIFORNIA SAN DIEGO
SAN DIEGO STATE UNIVERSITY

Photonic Transfer in DNA Nano Construct

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

in

Engineering Sciences (Bioengineering)

by

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2018

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Chair

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2018

DEDICATION

To my parents, Bahieh and Mohammad Reza

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ACKNOWLEDGEMENTS

First, 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 research idea and financial support to make my PhD experience productive. The joy and enthusiasm he has for the research was contagious and motivational for me, even during tough times in my PhD pursuit. I would like to thank the committee members, Professor Jan Talbot, Professor Xiaohua Huang, Professor Kee Moon, and Dr. Parag Katira. Their advice and comments helped keep me motivated in my research.

Chapter 3 is the reprint of some material currently being prepared for submission as it appears in Golkar Narenji, A., Heller, M., Efficient long-range fluorescence energy transfer in multiple donor single acceptor insulated DNA Nano construction. The author is the primary researcher/author of this work.

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Journal Articles:

- [1] Noah Goshi, A.Golkar Narenji, Sam Kassegne, Investigation of Effects of Nucleotide Content on Electrical Characteristics of DNA Plasmid Molecular Wire, accepted in Transactions on NanoBioscience Journal, July 2016.
- [2] S. Kassegne, D. Wibowo, J. Chi, V. Ramesh, A. Golkar Narenji, “AC Electrical Characterization and Insight to Charge Transfer Mechanisms in DNA Molecular Wires through Temperature and UV Effects,” published in IET Nanobiotechnology Journal, Jan 28, 2015.
- [3] V. Ho, A. Golkar Narenji, Lawrence Kulinsky and Marc Madou, “The Detachment Process and Operation Efficiency of Polypyrrole/Gold Bilayer Actuators,” published in JMEMS Journal, May 15, 2015.
- [4] A. Golkar Narenji, Michael Heller, Electronic and Photonic Transfer in DNA Nano Construct, to be submitted to the journal of Nanomedicine.
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- [2] A. Golkar Narenji, N. Goshi, S. Kassegne, “Effect of Temperature and UV light Illumination on Charge Transfer Mechanisms in DNA Molecular Wires”, SPIE Smart Structures Conference, March 8-12, 2015.

ABSTRACT OF THE DISSERTATION

Electronic and Photonic Transfer in DNA Nano construct

by

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Doctor of Philosophy in Engineering Sciences (Bioengineering)

University of California San Diego, 2018

San Diego State University, 2018

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A significant amount of work has been done to improve design and fabrication of DNA constructs with photonic and electronic transfer properties. In this thesis, we evaluated the prior work for both electronic and photonic transfer. While DNA constructs with first-order fluorescent resonant energy transfer (FRET) properties have proven useful, incorporation of

higher-order FRET and electronic properties into DNA has not yet led to any viable applications. In this thesis, we generated 35 base pairs long double-stranded (ds) DNA structures with different arrangements of five TAMRA donor dyes and a single TexasRed acceptor. In these constructs the distance of the distal donor dyes to the acceptor is greater than 1.7 nm or five base pairs (which is beyond the optimal FRET distance). The average FRET efficiency of these double stranded systems based on the donor intensity change and the acceptor-to-donor ratio of intensity change was 66% and 26%, respectively. Addition of surfactants and metal cations reduced quenching and enhanced the FRET efficiency of these DNA structures. After adding the surfactant and metal cations, the average FRET efficiency of these ds-systems based on the donor intensity change and the acceptor-to-donor ratio of intensity change was 89% and 75%, respectively.

We also reviewed the conductivity properties of DNA and how it is influenced by temperature, UV illumination and GC content. Results from literature indicate that temperature significantly changes DNA conductivity. Moreover, the UV exposure experiments indicate a decrease in DNA conductivity due to damage of GC base pairs and the phosphate group. We also investigated the effect of nucleotide content on DNA conductivity and we showed that the higher GC content results in higher conductivity.

Chapter 1

Introduction

DNA-based applications are useful in molecular computing, sensing, electronic, photonic, biosynthetic, drug delivery, top-down photolithographic, and bottom-up self-assembly nanofabrication [1]. The small size of DNA (diameter of 2nm, with 0.34nm separation between bases) and its unique self-assembly and self-replicating properties make it a strong candidate for nano electronic and photonic devices. An important drive of DNA nanotechnology research has been the development of DNA-based photonic and electronic wires and switches [2]. Photonic wires function through Förster resonance energy transfer (FRET) forces; FRET is a prevailing technique used to study the structure of biomolecules. The way in which FRET transfer is harnessed to turn DNA strands into photonic conductors is by the sequential transfer of a fluorescent signal from one site created on the DNA strand, a “donor”, to another modified DNA base, an “acceptor”. The schematic of the DNA molecule with fluorophores as one donor and one acceptor are shown in Figure 1.1.a. In some cases, the chemiluminescent group is used as a donor, as shown in Figure 1.1.b. Figure 1.2 is a schematic of photonic wires which shows extended energy transfer from donor to acceptor, where five donors are linked to a polynucleotide by linker arms and one acceptor is linked to the polynucleotide [3] .

In FRET, the donor absorbs photonic energy at one wavelength. Through a non-radiative dipole coupling process, the donor transfers energy to an acceptor which reemits the photonic energy in longer wavelength [4][5][6] as shown in Figure 1.3. Critical factors for energy transfer are (i) distance between donor and acceptor, (ii) fluorescence wavelength, which includes the excitation and emission peak of donor and acceptor, (iii) orientation of transition dipole, and (iv) life time of fluorophores in the excited state.

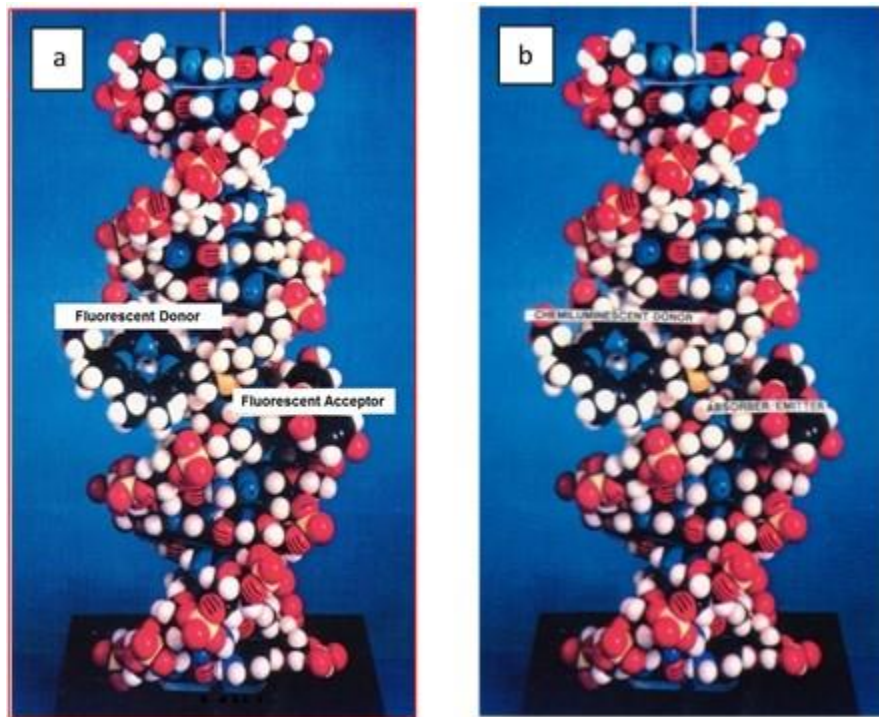


Figure 1.1: Chemiluminescent and fluorescent probes in DNA structure.[7]

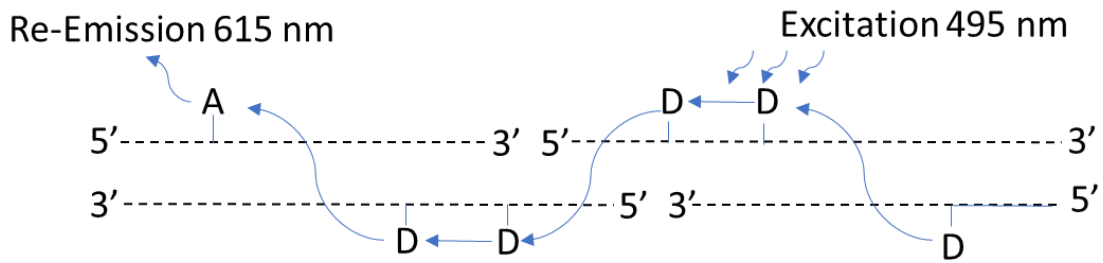


Figure 1.2: Multiple Donors to Single Acceptor in DNA Hybrid System.

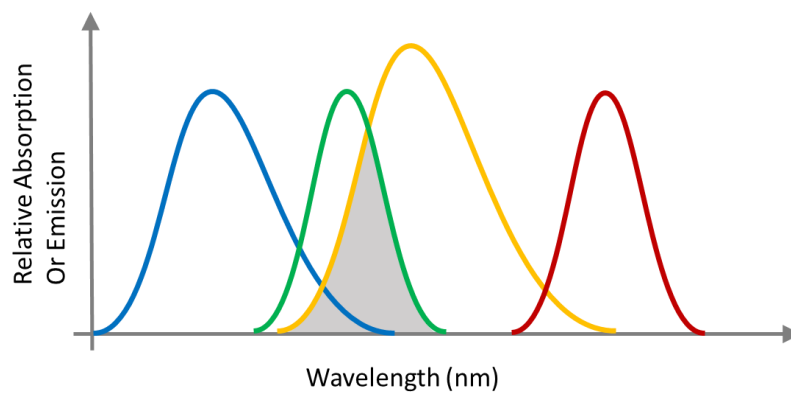


Figure 1.3: Relative absorption or emission with respect to wavelength [7]

DNA applications, including molecular sensors and clinical diagnostics, use DNA modified with either dyes, nanoparticles, or functional groups. In molecular sensors, the fluorescence signals of the dyes change due to DNA probe hybridization. However, if two dyes are in proximity, they will interact, resulting in dye dimerization. This causes significant quenching of fluorescent emissions, and thus will significantly lower the FRET efficiency [8]. To overcome this limitation, Heller *et al* showed that decoration of DNA with surfactants and divalent metal ions caused insulation of DNA structure, which reduces dimerization and quenching [8].

Major advantages of the FRET approach are: (i) extremely high sensitivity, (ii) excellent selectivity and (iii) wide dynamic range of fluorescence measurements[9]. FRET has been recognized as powerful tool to study G-quadruplexes due to its high sensitivity and multidimensionality. Different aspects of the molecular structure can be obtained as well as information on the concentration, binding events, and inter strand motion. With FRET technique, 1×10^{10} M strand concentration could be detected. The FRET approach also identifies ligands which target G-quadruplex DNA. G-quadruplex have shown anticancer activity because G-rich sequences which are found in genome are linked to mechanism relate to cancer, HIV, and other disease. FRET approach studies the ligand binding affinity in the presence of competitor and it could detect labeled strand in the presence of large excess of unlabeled oligonucleotide due to its selectivity. Juskowiak *et al.* also reported a FRET sensor based on quadruplex formation that was suggested for potassium detection. As an oligonucleotide probe, they used fluorescein as an acceptor and cationic conjugated polymer (CCP) as an external energy donor which has many fluorophores and it could measure wide range of florescence. In the absence of K^+ the probe exhibited inefficient FRET, whereas adding KCL results in the formation of a G quadruplex which caused an increase in FRET signal (about 16-times) of the acceptor. FRET is also advantageous for transmitting the information because it is not diffraction-limited, happening through-space over nanometer distances, and on the time scale of nanoseconds or less.

With regard to the electronic properties of DNA, the most widely used DNA-based nanotechnology is molecular wires[10][11][12][13][14][15]. For example, the latest reported transistor feature size in the semiconductor industry is IBM's 7nm FinFET logic chip. This uses silicon-germanium as the channel material, which is still at least two or three generations away

from 2nm feature size of potential DNA-based nanoelectronics. However, commercial transistors with IBM's 7nm sizes are still several years away with current feature sizes limited to 14nm. Therefore, the search continues for potential nanoelectronics-enabling materials and nano-fabrication techniques.

While DNA molecules continue to carry great promise for nanoelectronic devices, there are still many conceptual and technical challenges. These include conclusions about conductivity ranges, based on both experimental and theoretical research findings, that are contradictory and diametrically opposed [16]. Also, the electrical conductivity and charge transfer mechanisms of DNA-based molecular wires is still not well-understood, and the lack of DNA construct stability under various extreme environmental conditions are preventing significant progress in their viability.

In the first case, with respect to conductivity, theoretical models generally predict conductive behavior that is several orders of magnitude higher than what is shown by experimental results [17]. Typical theoretical models idealize DNA as a simplified 1D disordered system where disorderliness arises from base pair randomness[18]. This disorderliness leads to localization of charge carriers inside potential wells both in the lowest unoccupied and highest occupied molecular orbits (LUMO and HOMO) which affects drastically the electrical conduction through the molecule. Furthermore, while experimental research findings have suggested that conductivity is also a function of the type of base pairs involved, theoretical models have made no distinction between the various base-pair contents in nucleotides. In addition, in some of the theoretical and experimental models, critical parameters such as alternating and direct source of electrical current (AC & DC) are not

decoupled. Therefore, the lack of clarity in the electrical properties of DNA molecular wires continues.

Secondly, regarding charge transfer mechanisms, widespread experimental and theoretical studies over the past decade have presented different descriptions of charge transport mechanisms in DNA. In general, there has been progress in classifying these mechanisms under two main categories, the short-range electron tunneling from donor to acceptor through DNA[19][20][21][22][23] and the long range charge-hopping between discrete base orbitals[24][10][11]. Nonetheless, there still is significant disagreement on mechanisms that rule over short distance and medium-range transport mechanisms, due to the failure to incorporate effects such as DNA structure, thermal motion of charges, cations in solution, inter-molecular and intra-molecular attraction and repulsion, and influence of contacting conductors [25].

Thirdly, long-term stability has been a major concern in organic and molecular electronics where the stability is affected by such external factors like humidity, temperature and UV illumination.

Directly incorporated electronic and photonic functional properties allow connections to be formed within large organized structures which are created by self-assembly. The combination of the properties allows the creation of useful photonic and photovoltaic devices, amplification mechanisms, antenna arrays, DNA biosensors, and DNA diagnostic assay systems.

1.1 Dissertation Structure

This dissertation is mainly focused on electronic and photonic transfer in DNA nanoconstruct. Consequently, Chapter 1 is devoted to introduction on electronic and photonic transfer in DNA nanoconstruct.

In chapter 2, we have reviewed the electronic and photonic transfer in DNA nanoconstruct. In this chapter, we reviewed a significant amount of work which has been done to improve design and fabrication of DNA constructs with photonic and electron transfer properties. While DNA constructs with first-order fluorescent resonant energy transfer (FRET) properties have proven useful, however, incorporation of higher-order FRET and electronic properties into DNA has not yet led to any viable applications. The goal of this review is to evaluate the prior work and determine which approach might lead to successful application. By considering the advantages and disadvantages of each approach, we will evaluate whether technical or fundamental design issues are what limits successful applications for DNA photonic and electronic transfer.

Chapter 3 is on efficient long-range energy transfer in multiple donors and single acceptor insulated nanostructures. In this chapter, we showed that the long-range FRET efficiency in 35mer ds-DNA structures with different arrangements of five TAMRA donor dyes and a single TexasRed acceptor where 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) is highly efficiently. The average FRET efficiency of these ds-systems based on the change of donor intensity and based on ratio of intensity change of acceptor to donor are 66% and 26% respectively. Addition of surfactants and metal cations reduced quenching and enhanced the FRET efficiency of these DNA structures. Negatively charged SDS surfactant does not reduce

dimerization and emission quenching, addition of magnesium cations (Mg^{2+}) or sodium cations (Na^+) lead to a significant reduction in dimerization and emission quenching and produce higher FRET efficiency. After adding the surfactant and metal cations, the average FRET efficiency of these ds-systems based on the change of donor intensity and based on ratio of intensity change of acceptor to donor are 89% and 75% respectively. Antenna effect for all different arrangements of five donors and a single acceptor has been calculated and compared with the control sequences. We also investigated the short-range energy transfer in DNA Nano construct for 2 TAMRA donors and a single TexasRed acceptor.

In the next chapter, chapter 4, we studied the effect of temperature, UV illumination and DNA GC content on charge transport mechanisms in DNA. Research into the use of DNA molecules as building blocks for nanoelectronics as well as nanosystems continues. The conductivity of DNA molecules depends on many factors including not only the structure and the surrounding chemical environment, but also the interaction with the substrate surface. Based on the literature, the data from temperature results indicates significant change in DNA conductivity and the UV exposure experiments indicates decreased conductivity of λ -DNA molecular wires after UV exposure. We also reviewed the effect of nucleotide content on the conductivity of DNA molecular wires which shows that the higher GC DNA content shows higher conductivity.

Finally, the dissertation is concluded with the summary of the presented work in the chapter 6 along with the brief future works that one may consider as an extension to this dissertation research pathway.

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Chapter 2

Review on Electronic and Photonic Transfer in DNA Nano Construct

2.1 Introduction

DNA-based applications are useful in molecular computing, sensing, electronic, photonic, biosynthetic, drug delivery, top-down photolithographic, and bottom-up self-assembly nanofabrication [1]. An important drive of DNA nanotechnology research has been the development of DNA-based photonic and electronic wires and switches [2]. Photonic wires function through FRET forces; FRET is a prevailing technique used to study the structure of biomolecules. The way in which FRET transfer is harnessed to turn DNA strands into photonic conductors is by the sequential transfer of a fluorescent signal from one site created on the DNA strand, a “donor”, to another modified DNA base, an “acceptor”.

In FRET, the donor absorbs photonic energy at one wavelength. Through a non-radiative dipole coupling process, the donor transfers energy to an acceptor which reemits the photonic energy in longer wavelength[3][4][5][6][7].

DNA applications, including molecular sensors and clinical diagnostics, use DNA modified with either dyes, nanoparticles, or functional groups. In molecular sensors, the fluorescence signals of the dyes change due to DNA probe hybridization. However, if two dyes are in proximity, they will interact, resulting in dye dimerization. This causes significant quenching of fluorescent emissions, and thus will significantly lower the FRET efficiency [8]. To overcome this limitation, Heller *et al* showed that decoration of DNA with surfactants and divalent metal ions caused insulation of DNA structure, which reduces dimerization and quenching [8].

Major advantages of the FRET approach are: (i) extremely high sensitivity, (ii) excellent selectivity and (iii) wide dynamic range of fluorescence measurements[9].

With regard to the electronic properties of DNA, the most widely used DNA-based nanotechnology is molecular wires[10][11][12][13][14][15]. While DNA molecules continue to carry great promise for nanoelectronic devices, there are still many conceptual and technical challenges. These include conclusions about conductivity ranges, based on both experimental and theoretical research findings, that are contradictory and diametrically opposed [16]. theoretical models generally predict conductive behavior that is several orders of magnitude higher than what is shown by experimental results [17]. Typical theoretical models idealize DNA as a simplified 1D disordered system where disorderliness arises from base pair randomness[18]. Furthermore, while experimental research findings have suggested that conductivity is also a function of the type of base pairs involved, theoretical models have made

no distinction between the various base-pair contents in nucleotides. Therefore, the lack of clarity in the electrical properties of DNA molecular wires continues. Also, the electrical conductivity and charge transfer mechanisms of DNA-based molecular wires is still not well-understood. The charge transfer mechanisms are mainly classified under two main categories, the short-range electron tunneling from donor to acceptor through DNA[19][20][21][22][23] and the long range charge-hopping between discrete base orbitals[24][10][11][25]. Furthermore, the lack of DNA construct stability under various extreme environmental conditions are preventing significant progress in their viability. long-term stability has been a major concern in organic and molecular electronics where the stability is affected by such external factors like humidity, temperature and UV illumination.

2.2 Energy transfer in DNA construct

In this section, we will review (i) energy transfer mechanisms and models, (ii) energy transfer through one donor and one acceptor, (iii) energy transfer through multiple donors and one acceptor, and (iv) FRET application with DNA.

2.2.1 Energy transfer mechanism and model

FRET is a technique used for measuring the distance between two molecules conjugated to different fluorophores. Lee et al. did accurate FRET (independent of instrumental factors, such as excitation intensity or detector alignment) measurements within single diffusing biomolecules, using confocal microscopy equipped with alternating-laser excitation for detection (ALEX) [26]. This device is useful because it uses corrections that account for cross-talk terms that contaminate the FRET-induced signal, and for differences in the detection efficiency and quantum yield of the probes. They showed accurate FRET. ALEX could benefit structural analysis of inaccessible biomolecules do to their heterogeneity or transient nature.

Gordon et al. used fluorescence microscopy to obtain quantitative temporal and spatial information about the binding and interaction of proteins, lipids, DNA, RNA, and enzymes[27]. The method they used is corrected for cross talk terms and for the dependence of FRET on the concentrations of the donor and acceptor.

In the mechanism of FRET between one donor and one acceptor, the donor initially absorbs the energy due to excitation of incident light and it transfer its excitation energy to a nearby acceptor in a non-radiative fashion through long-range dipole-dipole interactions [4]. Energy transfer manifests itself through decrease in the donor fluorescence followed by an increase in acceptor fluorescence intensity. In the molecular level, the absorption of a light by a fluorophore induces a rearrangement of the electronic structure of the molecule and stores some energy at the excited state. After a short period of time, the electronic structure of the molecule decreases back to its equilibrium state (the ground state). The relaxation of the excited state to its ground state happens with the emission of a photon of fluorescent light at a lower energy than the light originally absorbed. The mechanism behind the energy transfer between multiple donors and one acceptor starts after excitation, where the first donor absorbs the energy at one wavelength. The first donor then passes on the energy to the next donors before passing the energy to the acceptor. The acceptor is then reemitting the energy at higher wavelength.

The rate of energy transfer from excited donor to acceptor can be given in the following form [28].

$$K_T = \text{const } J n^{-4} R^{-6} k^2 \quad \text{Equation (1)}$$

K_T is a rate of energy transfer from the excited donor to the acceptor, R is the distance between donor and acceptor chromophores, J represents the spectral overlap integral, which is proportional to the overlap of the emission spectrum of the donor and the absorption spectrum of the acceptor, k^2 is an orientation factor, and n is the refractive index of the medium.

Efficiency (E) of energy transfer can be written as [29].

$$E = \frac{K_T}{K_T + K_F + K_D} \quad \text{Equation (2)}$$

K_F is the rate constant of fluorescence emission of the donor and K_D is the sum of the rate constants of all other de-excitation processes of the donor.

As shown in Figure 2.1, FRET efficiency is determined by a $1/R^6$ distance dependency relationship [8].

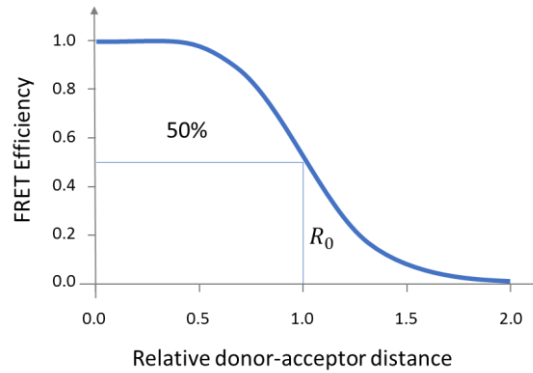


Figure 2.1: Dependence of energy transfer on distance. R_0 is the Förster radius.

The formula shows that the rate of energy transfer is inversely proportional to the sixth power of the distance between the donor and acceptor. Therefore, at distances larger than the Förster radius, the efficiency of the transfer rapidly drops to zero. R_0 is the Förster radius at which half of the excitation energy of the donor is transferred to the acceptor chromophore. Therefore, Förster radius (R_0) is referred to as the distance at which the efficiency of energy transfer is 50%.

R_0 depends on the fluorescence quantum yield of the donor in the absence of the acceptor (f_d), the refractive index of the solution (n), and the spectral overlap integral of the donor-acceptor pair (J) and is given by[4].

$$R_0 = 9.78 \times 10^3 (n^{-4} \times f_d \times J)^{1/6} \text{Å}^0 \quad \text{Equation (3)}$$

2.2.2 Energy transfer through one donor and one acceptor

Ha et al. demonstrated that energy transfer can be measured on a single donor-acceptor pair.

They were being able to monitor conformational changes such as rotations and distance changes of biological macromolecules in nanometer scale [30]. Heller et al., as shown in Figure 2.2., also showed the energy transfer between single donor-acceptor pair. They used TAMRA conjugated DNA as a donor and TexasRed conjugated DNA as an acceptor [8]. When hybridization of the TAMRA conjugated DNA strand with the TexasRed conjugated DNA strand brings the donor and acceptor dyes into close proximity, the high FRET efficiency is expected. However, they showed that this efficiency is not high enough as the energy transfer is quenched due to dimerization between dyes. This can be resolved by adding surfactants and divalent metal ions. Cationic surfactant like cetyl-trimethylammonium bromide (CTAB) decreases the dimerization by neutralizing the negatively charged double-stranded DNA (ds-

DNA). A negatively charged surfactant like SDS does not reduce dimerization and emission quenching because they have the same negative charges as the DNA. However, as shown in Figure 2.4(b), the addition of sodium cations (Na^+) and magnesium cations (Mg^{2+}) leads to a significant reduction in dimerization and emission quenching. This produces higher FRET efficiency. The addition of a neutral surfactant (e.g. Triton X-100) does not reduce dimerization and emission quenching in the FRET ds-DNA structures.

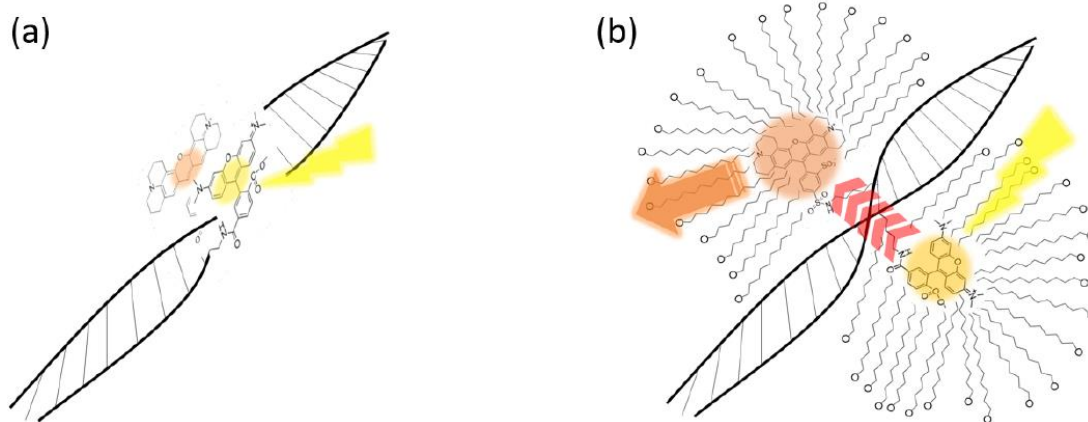


Figure 2.2: Schematics on (a) quenched emission by dimerization without surfactant and (b) enhanced emission by FRET with surfactant in dye-conjugated DNA

2.2.3 Energy transfer through multiple donors and one acceptor

An important drive of DNA nanotechnology research has been the development of DNA-based photonic wires and switches that operate via FRET. In these models, double-stranded DNA is used as a template to organize a linear array of fluorophores to accept a photon input at one terminus and emit a photon output at the opposite terminus. Excitonic energy is transferred nonradiatively along the length of photonic wires and between different

fluorophores aligned from shortest to longest wavelengths of absorbance and emission. For example, early work by Kawahara et al. demonstrated energy transfer along a DNA wire with three different fluorophores over ~ 8 nm [31]. Ohya et al. and Heilemann et al., as seen in Figure 2.3, demonstrated energy transfer with five different fluorophores over distances of ~ 13 nm [32][33]. Spillman et al. did this with seven fluorophores over >16 nm [34]. Hannestad presented longer energy transfer over a distance of more than 20 nm [35]. As a result, studies showed that the energy could transfer between 1 donor to 9 donors.

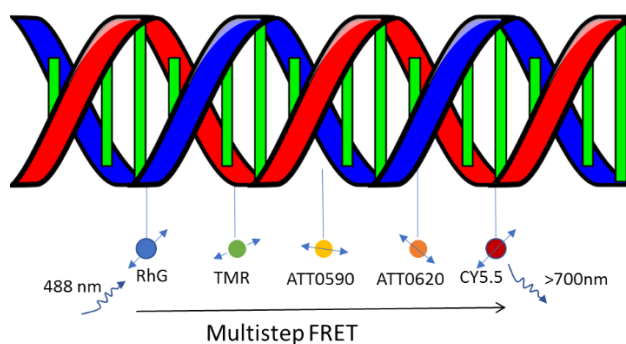


Figure 2.3: Energy transfer between five different fluorophores [36]

For antenna, photonic amplification system, biosensors, homogeneous and heterogeneous DNA diagnostic systems the ratio of donor: acceptor should be more than one. Previous workers [5] have proposed for antenna or photonic amplification applications, the number of donors to acceptor could range from the lower limit of 2:1 to as high as 10^6 :1. When using heterogeneous DNA diagnostic and biosensor applications, the number of donors to an acceptor could range from the lower limit of 2:1 to upper limit of 10^5 :1. For homogeneous DNA

diagnostic applications, the ratio of donors to acceptor could range from lower limit of 2:1 to upper limit of 10^4 :1.

Regardless of knowledge of the optimal distance between DNA modifications [8], it is still hard to have the ideal conformation of the DNA-organized FRET-based photonic wires. Some of the difficulties come from the photobleaching and dimerization of dyes. The dye dimerization is often common in the DNA with multiple donors. However, as seen in Fig 2.4, this can be resolved by adding surfactants to the DNA as mentioned above.

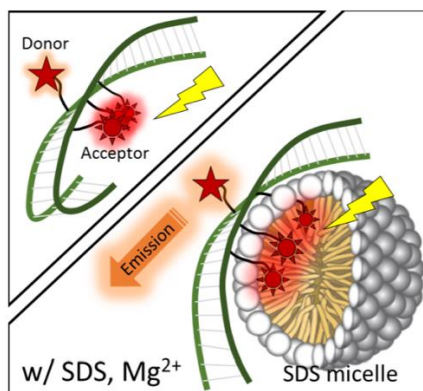


Figure 2.4: Schematics of three TAMRA donors and one TexasRed acceptor on ds-DNA structures both with and without SDS micelle and cations.

2.2.4 FRET application with DNA

Photosynthesis, the basis of all life on Earth, converts solar energy into chemical energy. Photosystems are tightly packed in the thylakoid membrane with several hundred antenna chlorophylls and accessory pigments surrounding a photoreaction center[37]. First, the absorption of the photon excites an antenna molecule (chlorophyll molecules or accessory pigments) which called donor and raise the electron to a higher energy level. Then excited antenna molecule passes the energy to neighboring chlorophyll molecule which called resonance energy transfer. Here the energy transfer happens from higher energy pigments (blue

absorption) to lower energy pigments (red absorption). This energy is then transferred to a reaction center (acceptor) as shown in Figure 2.5. The excited reaction center passes an electron to an electron acceptor. The electron hole in the reaction center is filled by an electron from an electron donor. Photochemical reaction in reaction center converts the energy of a photon to separation of charges initiating an electron flow. Photosynthesis helps chemists to design artificial systems which mimic every aspect of it.

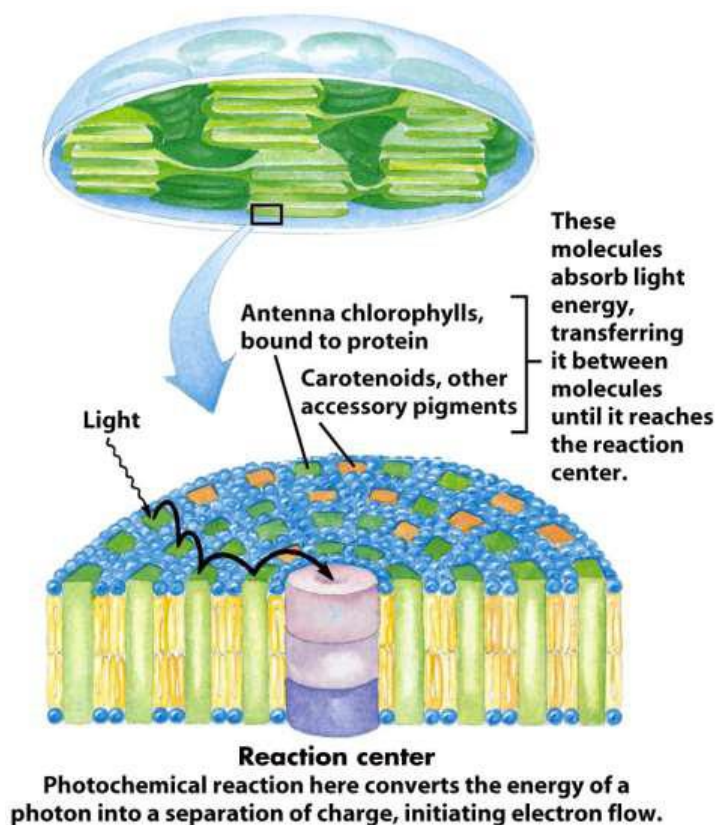


Figure 2.5: Organization of photosystems in the thylakoid membrane

DNA-based FRET probes have a wide variety of applications both *in vivo* and *in vitro*[28]. Some of these applications monitor many biochemical reactions such as

polymerization, recombination, and ligation of DNA. The DNA-based FRET probes also can be used to detect protein mutations and DNA structural transitions.

Green fluorescence protein (GFP) could be used as a marker for gene expression and structural protein localization in living cells. blue fluorescent protein (BFP) could be used as a marker for mutant protein. Figure 2.6 illustrates the strategy for detection of protein-protein interactions using FRET. Two proteins are labeled one with BFP (the donor) and the other with GFP (the acceptor). If two proteins are in close contact with each other the increased intensity at the acceptor emission will be observed. No interaction between the proteins may results in no GFP emission.

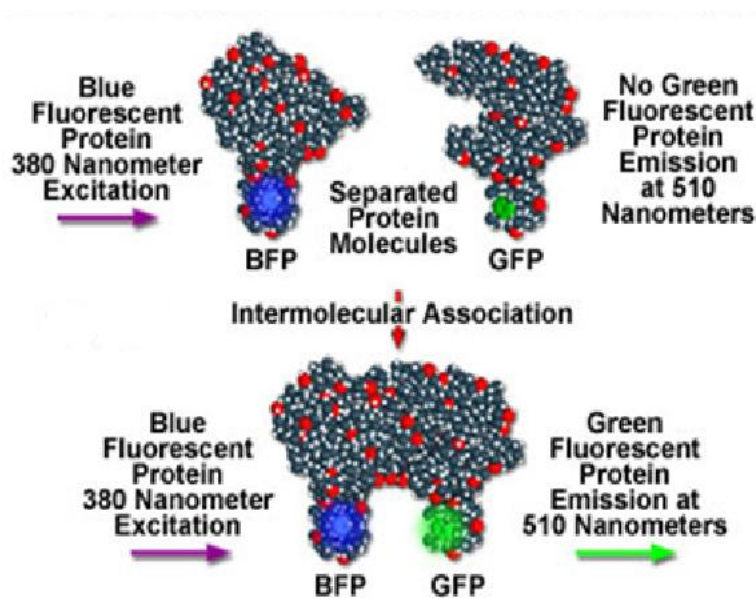


Figure 2.6: FRET detection of in-vivo protein-protein interactions

Another application of these probes involves the ability of DNA to exhibit structural flexibility, forming tetraplex structures known as guanine-quadruplexes or G-quadruplexes (also called G-tetrads) as shown in Figure 2.7. FRET technique can investigate the structural

transitions of these G-quadruplexes. G-quadruplexes have recently received great attention because G-rich sequences, often found in the genome, have potential links to mechanisms relating to cancer, HIV, and other diseases[38].

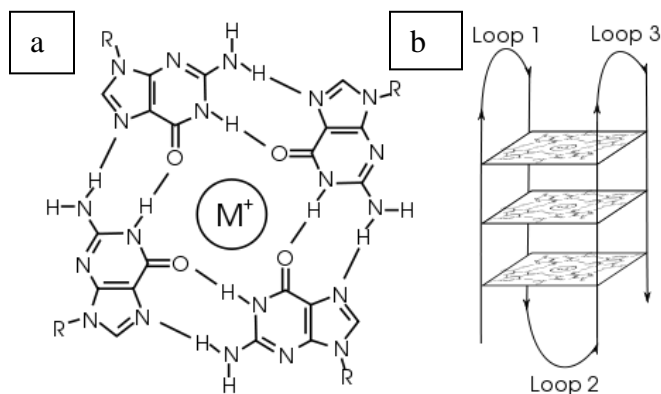


Figure 2.7: a, Structure of G-tetrad showing hydrogen bonds between four guanines and the interaction with a cation (filled circle). b, An intramolecular G-quadruplex.

FRET techniques are also used for studies of DNA hybridization. When two oligonucleotides labelled with a donor and an acceptor, respectively, are close to each other, FRET can occur as shown in Figure 2.8. Two signals are received in the probe. One is from the hybridized DNA, and the other is from the non-hybridized DNA. This allows the real-time observation of the DNA hybridization. One benefit of this application is that neither solid support nor washing is needed. The rationale behind the adjacent hybridization probes is when the FRET system is formed by hybridization, the quenching of the donor and the sensitization of the acceptor fluorescence could be seen. However, the rationale behind the molecular beacon is when the probe is not hybridized to the target, the donor and acceptor are brought in proximity by the stem formation, which results in the FRET-based quenching of the donor. However, in the presence of the target sequence, the probe forms longer and stronger hybrids with the target

than by forming the stem. As a result, the FRET transfer is disturbed which results in the appearance of the donor fluorescence.

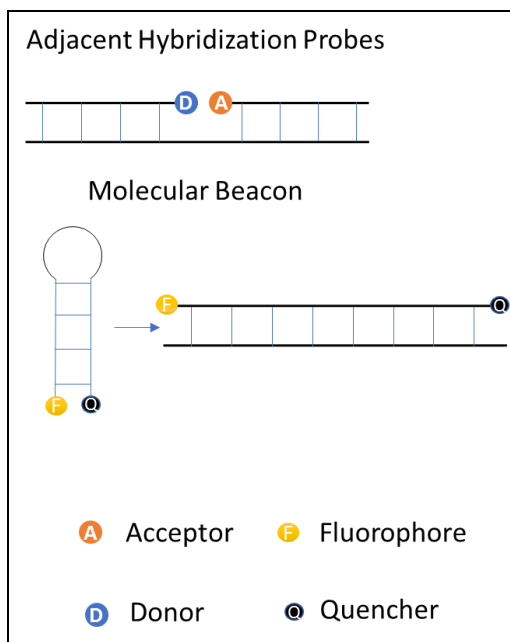


Figure 2.8: Adjacent hybridization probe

Similarly, Heller et al. showed that FRET can be used to study homogeneous hybridization systems [7]. They showed that at high temperatures (40°C) there is no energy transfer, since there is no hybridization-in this temperature, whereas the energy transfer is maximum at low temperature (5°C) when the hybridization is maximum[7].

Furthermore, Zhang et al. reported an ultrasensitive nanosensor based on FRET that can detect low concentrations of DNA in a separation-free format. This organic/inorganic hybrid FRET nanosensor produces an extremely low level of background fluorescence, which is difficult to achieve with conventional organic FRET probes. This system used quantum dots (QDs) linked to DNA probes to capture DNA targets [39][40][41][42][43][44]. Figure 2.9 shows the

assembly of QDs and FRET between Cy5 as an acceptor and a QD as a donor in a nanosensor assembly.

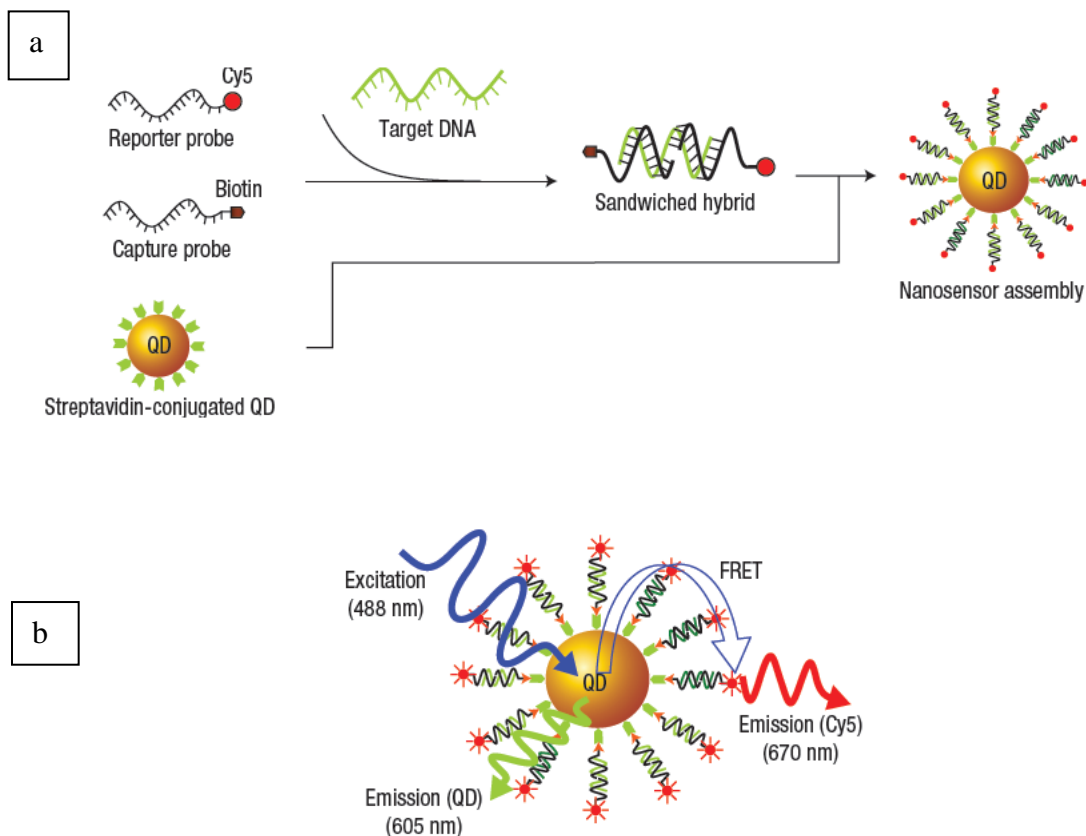


Figure 2.9: Schematic of single-QD-based DNA nanosensors. a, Conceptual scheme showing the formation of a nanosensor assembly in the presence of targets. b, Fluorescence emission from Cy5 on illumination on QD caused by FRET between Cy5 acceptors and a QD donor in a nanosensor assembly.

Figure 2.10 shows DNA nanostructures that deliver a useful tool for the organization of photonic components either in a linear fashion or in branched networks. With the addition of plasmonic nanostructures, semiconductors, and proteins, more advanced molecular circuits can be built [8].

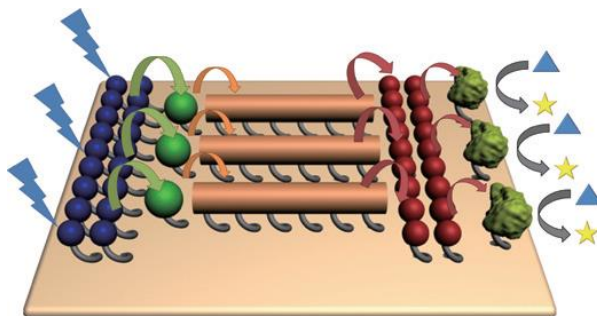


Figure 2.10: DNA nanostructures provide a useful tool for the organization of photonic components in a linear fashion or in branched networks. The modularity of assembly, along with the plethora of DNA functionalization of photonic components, allows for the construction of photonic molecular circuits. Light-harvesting complexes can be spatially clustered and aligned, where sequential energy or charge-transfer processes lead to optimized channeling 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[46].

2.3 Electronic transfer in DNA

2.3.1 DNA Electrical Conductivity Mechanisms and Models

Based on the wide body of work in the literature, charge transfer (CT) mechanisms in DNA can be divided into two main transport models: short-range electron tunneling from donor to acceptor through DNA[19] [21][23][22] and long-range charge hopping between discrete base orbitals[10][11][24] (Figure 2.11). In the single-step electron-tunneling CT mechanism, current theoretical models assume DNA molecule to behave as a one-dimensional aromatic crystal with π -electron conductivity[47][48] In electron tunneling, electrons show a wavelike property and the resistance increases exponentially with DNA length. However, in charge hopping, the charge losses its wavelike properties and the resistance increases linearly with the length [49]. Moreover, in another mechanism called phonon-assisted polaron, the injection of charge disturbs the

molecular structure which results in intra-base distance reduction (Figure 2.12). This will then, subsequently, give way to increased π -electron overlap[50]. Generally, in the hopping mechanism, the G base is considered the first candidate to hop, because it has the least ionization potential among the four bases.

Different groups have different ideas on charge transfer in DNA. Barton and coworkers suggested long-range electron transfer in DNA[31][51][25]. When the donor intercalated onto the DNA was photo-excited, the fluorescence of that donor was quenched because of electron transfer to the acceptor. Barton, et al. showed very rapid transfer of carriers through π -stacked base pairs, even though reproduction of the results with other donors and acceptors was found problematic[52]. The study by Livshits et al., in which transport in a rigid guanine tetrad wire laid directly on the surface was measured, supports that charge transport occurs via a thermally activated long-range hopping between multi-tetrad segments of DNA[14]. However, studies by Xiang, et al. show that something intermediate is occurring that is not exactly hopping, because the electron still displays some of the wave properties and the resistance increases with the DNA length in the oscillation manner.

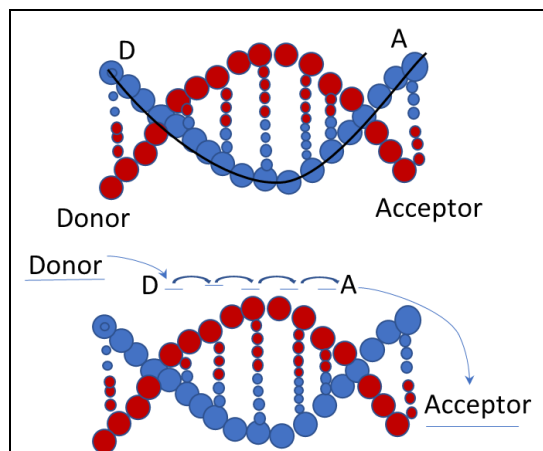


Figure 2.11: Two possible charge transport mechanism through DNA, namely, electron-tunneling through phosphate back-bone, and charge hopping through discrete molecular orbitals of bases.

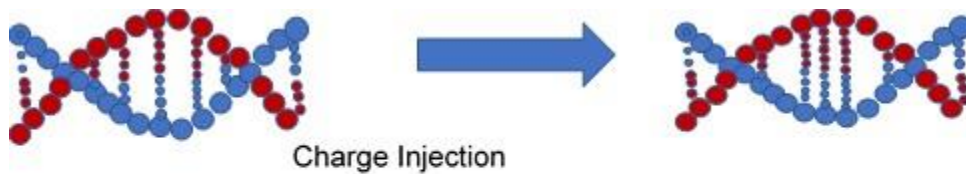


Figure 2.12: Phonon-assisted polaron hopping mechanism where the injection of the charge causes reduction of intra base distance and consequently more overlapping between the π orbitals.

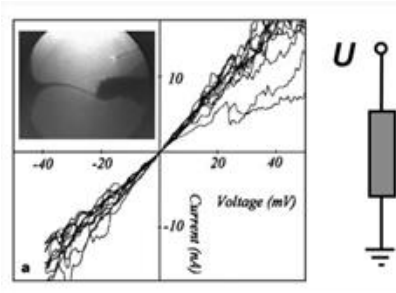


Figure 2.13: I–V curve taken for a 600-nm-long DNA rope. In the range of ± 20 mV, the curves are linear; above this voltage, large fluctuations are apparent. From the linear dependence at low voltage we derive a resistance of about $2.5 \text{ M}\Omega$ [53].

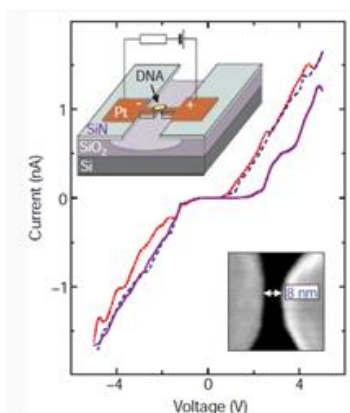


Figure 2.14: Current–voltage curves measured at room temperature on a DNA molecule trapped between two metal nanoelectrodes.

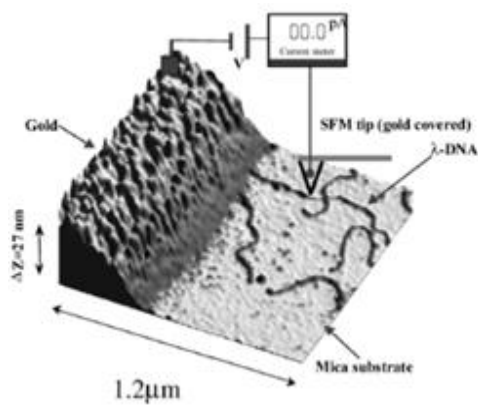


Figure 2.15: Three-dimensional SFM image of the channel border, showing two DNA molecules in contact with the left gold electrode. The image size is $1.2 \text{ mm} \times 1.2 \text{ mm}$. Scheme of the electrical circuit used to measure the DNA resistivity has been shown [54].

However, the ideal CT model should consider additional factors such as effect of DNA structure, thermal motion of charges, effects of environment such as cations in solution, temperature, UV light, inter-molecular and intra-molecular attraction and repulsion in DNA, DC, AC source and so forth.

With respect to conductivity, different groups have shown varied results for DNA Conductivity. The work by Fink et al [53] reported conductivity behavior in DNA (Figure 2.13) [55]. As shown in Figure 2.13, they used direct measurements of electrical current as a function of the potential applied across a 600 nm long DNA rope, which shows efficient conduction through the DNA rope. The 1-DNA molecules are placed onto a regular array of 2- μ m holes in a carbon foil and the sample holders has been covered by gold electrode. Porath et al. also used the direct current measurements and they reported semi conductive behavior[56] where the DNA molecule (30 base pairs, double-stranded poly(G)-poly(C)) is 10.4 nm long, and the metal nanoelectrodes are separated by 8 nm (Figure 2.14). The work by de Pablo et al also used current-voltage (I-V) measurements through DNA molecules adsorbed on mica which shows insulator behavior[54] as shown in Figure 2.15. The last method is not a good method to truly measure the conductivity as the mica surface may influence DNA conductivity. The first method is the best as they used vacuum which could eliminate all other currents from artifacts.

Moreover, it should be noted that-the current research studies have been predominantly limited to theoretical analysis that is mainly focused on a 1-dimensional conduction modeling, with few and far in between the experimental works [57]. In next section, we review the conductivity under DC electric current, AC electric current, effect of temperature, UV on conductivity of DNA.

2.3.2 Theoretical Conductivity Models in DNA Molecular Wires

Currently, as mentioned earlier, there is a clear trend emerging towards accepting a few key mechanisms as the most plausible CT mechanisms; namely, single-step short-range quantum tunneling and long-range thermally activated charge hopping. However, the effect of parameters such as temperature-dependent behavior, DC and AC source, UV irradiation, on conduction have not been explained very well by these mechanisms. Moreover, it should be noted that, current research studies have been limited to predominantly theoretical analysis that is focused mainly on a 1-dimensional conduction modeling, but there have been very few experimental works. In next section, we review the conductivity under DC electric current, AC electric current, effect of temperature, UV and E-Field on conductivity of DNA.

2.3.2.1 Conductivity of DNA under DC Current – Theoretical Model

In theoretical approaches where DC mode is considered, conductivity - where the electrons travels through sugar base-pairs via tunneling - is generally found to display an exponential dependence on the donor-acceptor distance as described by Eq. 4 and Eq.5 [58][59].

$$G = e^{-\beta L} \quad \text{Equation (4)}$$

Where G is conductivity, β is length decay factor, and L is the length of the DNA duplex.

$$\sigma_{DC} = \sum_{i=1}^4 \sigma_0 e^{-\Delta E_i / KT} \quad \text{Equation (5)}$$

Where σ_0 is electrical conductivity at infinite temperature, given constant and homogenous value of electron density in the LUMO, ΔE is thermally activation energy, K is Boltzmann constant and T is temperature[58]. The summation is done over 4 base-pairs. The equation shows that at a certain temperature, the free electrons can carry DC-electric energy by thermal drift motion through the bases. This motion is thermally activated as $e^{(\Delta E/kT)}$ which shows a semiconductor-like behavior of DNA. Moreover, this equation also shows the strong dependence of the electrical conductivity on high temperatures but, in contrast, weak temperature dependency at low temperature.

A study by Tran et al [18] shows a similar exponential trend and dependency on temperature as follows:

$$\sigma = \sigma_0 e^{-(\Delta/2kT)} \quad \text{Equation (6)}$$

Where $\sigma_0 = 1.9 \cdot 10^2 (\Omega\text{cm})^{-1}$ and $\Delta=0.33$ for dry λ -DNA [18].

2.3.2.2 Conductivity of DNA under AC Current – Theoretical Model

Although different models have been suggested for the conductivity behavior of DNA molecular wires under alternating current (AC) signals and its dependence on frequency[60][17][58][57], its electrical behavior under AC conditions has not been completely understood. Interestingly, the higher frequency response characterization of the DNA is of more importance, since it might open a path to the utilization/realization of the future ultra-thin (i.e., ~2nm) electrical components enabling ultra-dense high-speed circuits. Most of the theoretical models consider DNA as a disordered system with random base-pairs[61][62]. This dependency of

DNA conductivity on frequency, which is predicted to be strong by these models, is driven by the transfer of electrons between localized states. Application of AC electric field to the localized electrons stimulates and forces them to follow the applied field. The occurrence of localized electronic states in disordered systems was first noted by Anderson in 1958 [61], who argued that in a disordered medium, the exact wave functions are localized in a small region of space. This phenomenon was later reported by Mott [62] who proposed the 1-dimensional disordered system model known as Mott-Halperin Law for AC conductivity[17], [62]. Equation 4 is used to calculate the AC-conductivity when the electron transport occurs through hopping between DNA base pairs in the 1D disordered system. This equation was later developed to Equation 5 below, which calculates the AC-conductivity while taking temperature and frequency into account[58].

$$\sigma_{AC} = \omega^2 \ln^2\left(\frac{1}{\omega^2}\right) \quad \text{Equation (7)}$$

$$\sigma_{AC} = \sum_{i=1}^4 \sigma_L \frac{\omega^2 \tau_{0i}^2 e^{(\Delta E_i K T)^2}}{1 + \omega^2 \tau_{0i}^2 e^{(\Delta E_i K T)^2}} \quad \text{Equation (8)}$$

Where ω is frequency, σ is conductivity, τ is referred to as relaxation time, E is the electric field, K is the Boltzman constant, and T is temperature.

Another model by Rosenow [63] suggests that the AC conductivity in the 1D chain takes the form of:

$$\sigma_{AC}(\omega) \sim \omega^2 \ln^2\left(\frac{1}{\omega}\right) \quad \text{Equation (9)}$$

which follows the same law for semiconductor materials in solitons regime ; Fogler *et al* introduce four different regimes for semiconductor materials including solitons, 2-level systems, classical glass and nearly free chain regimes[17].

Moreover, alternating current conductivity could be related to DC conductivity, given as equation 7 where s is exponent factor, and it is highly temperature dependent and it can be found to be 0.68 using the Equation (8) where τ_{0i} is a limit value of the relaxation time of localized electrons; it corresponds to the relaxation time when temperature tends to infinite values. The maximum electrical conductivity occurs when there are no localized electrons in the LUMO i.e. when all electrons, in the band, participate in carrying the electrical currents [58].

$$\sigma_{AC} = \sigma_{DC} + \sigma_0 \omega^s \quad \text{Equation (10)}$$

$$\sigma = \ln\left\{ \sum_{i=1}^4 \left(\frac{\sigma_L}{\sigma_0} \right) \frac{\omega^2 \tau_{0i}^2 e^{(\Delta E_i / KT)^2}}{1 + \omega^2 \tau_{0i}^2 e^{(\Delta E_i / KT)^2}} \right\} / \ln(\omega) \quad \text{Equation (11)}$$

The reason the experimental value differs from the theory is that the theory does not consider conformational changes in-DNA structure that could cause by humidity, temperature, and-overall environmental conditions. Moreover, the theory does not take into account the order of the sequences that have an effect on DNA conductivity reported by many groups[49][64].

2.3.3. Experimental Results in DNA Conductivity

There is an increasing amount of literature on experimental investigations of conductivity in DNA [65][66][67][54][68][69][55]. In this section, we focus on some of the key findings and the measurement techniques employed. Some of the research in the literature reported divergent conductive properties of DNA molecular wires. In this section, we address the key experimental parameters that could account for these inconsistent behaviors.

Environmental conditions may have significant effects on DNA conductivity [70]. For instance, the presence of water creates resistivity, ionic current transfer and protonic transfer [71]. The results of Tuukkanen *et al.*[72] support the idea that there is ionic current transfer and inverse relationship between the humidity and the resistance. However, they reported the humidity to be important in maintaining the DNA structure. This will improve the electron transport mechanism through the overlap of π -orbitals of the base-pairs. Boxuan Shen *et al.* show that the water molecules also play a big role in DNA origami conductance[73]. Similarity, Dunlap Garcia *et al.* report insulating property for dry DNA and Tuukkanen *et al.* [72] report better conductivity for moist DNA molecular wire. However, Porath *et al.*[65] and Felicia & Porath *et al.*[70] [66]report insulating behavior even at 50% humidity. Future design could control for humidity by using vacuum condition where the vacuum make the molecule dried.

Another source of uncertainty comes from the possible electrical contact resistance created between electrodes and DNA molecular wires. Direct electrical measurement of DNA requires a good contact between the molecule and the probing electrodes. However ,with the current available

technology, making contacts with a single molecule is hard to perform and nearly impossible to control microscopically[74].

The substrate used often directly affects the conductivity of DNA and can mask the true conductivity of DNA molecular wires. Previous work has shown that substrates such as SiO₂ and mica give rise to insulator-type behavior in DNA molecular wires[72]. Kassegne et al showed that use of high aspect ratio chips (50-100 μm tall) can eliminate the effect of the substrate on DNA conductivity [70].

Moreover, a study by Barton et al shows that DNA hybridization plays an important role on the ability of DNA to conduct charge. Mutation in DNA may cause less π orbital staking between the base pairs which causes fewer electrons to transfer through the double helix DNA. As shown in Figure 8, In the case of fully matched DNA, the electrons could transfer through the double helix DNA through the base pairs π orbital stack route, which reduces the intercalated dye molecule (the redox indicator) tethered to the DNA strand and in turn reduces the ferricyanide solution that is the redox couple as shown in Figure 19. [75].

Kassegne et al report that DNA can be used as a molecular wire in nanoelectronic devices and the direct electrical measurement method they used shows the semiconductor behavior of DNA [76]. However, Porath et al show that G4-DNA, which can be used as a molecular wire, shows better conductivity than natural DNA which shows the currents of tens to over 100 pA [14]. However, other groups have shown that DNA can be used as a nanowire if coated with other conductive material such as CdS or metal. Braun et al coated λ -DNA with silver and found that the decorated DNA shows much higher conductivity, 7M Ω , than bare DNA, at 10 T Ω [13]. Similarly Aich et al. showed that M-DNA which is a complex of DNA with divalent metal ions

(Zn^{2+} , Co^{2+} , or Ni^{2+}) might possess unusual conductive properties as imino proton in each base pairs of duplex were substituted by a metal ion [77]

Currently, the programmed self-assembly of DNA strands via techniques such as "DNA origami" or "DNA bricks" provides the most precise method to construct nanoscale objects of predefined size, shape and composition[78]. DNA origami is the nanoscale folding of DNA to create variety of geometric shapes. This creation is because of the interactions between complementary DNA base pairs. DNA bricks are short synthetic DNA strand which creates 3D structures by self-assembly[79]. Conductance of DNA origami is very low in dry conditions, but becomes higher as humidity increases [80] Data analysis based on the IS also suggest that the water molecules play a big role in DNA origami conductance, which means the later developed 3D DNA origami constructions may have improved conductance since their compact inner structures can hold more organized water molecules.

However, Narenji et al. also showed that DNA molecular wires are not as stable as expected. They showed that over time, the DNA molecular wire will lose its conductivity. This is because of the effect of humidity over time and because of the change in DNA conformation and DNA hybridization[81][76].

2.3.4 DNA Electrical Conductivity as a Function of Base-Pairs

An increasing amount of research studies point towards the sequence dependency of DNA conductivity[22][82][83][84][85][86][64][87]. It has been reported that guanine has lower potential compare to other bases (see table 2.1); therefore, it is the first candidate to hop. Consequently, triple G has a lower ionization potential as it has more overlapping between its π -orbital compared to single or double G base-pair; Ratner and co-workers reported that the

ionization potentials of GG and GGG were lower than G by 0.5 and 0.7eV, respectively[49]. Work by Saito and colleagues established that the GGG sequence was easier to oxidize than G[64].

The sequence dependence of conductivity was demonstrated by measuring the charge transfer mechanism with regard to localization length[85]. The localization lengths of poly (dG) – poly (dC) DNA tend to infinity, meaning that the sequence is perfectly conducting compare to Lambda DNA with 45% GC. Similarly, Song *et al.* showed that the rate of charge transfer depends on the distance and sequence context of the DNA[86]. However, the work also showed that randomness may play a bigger role in DNA conductivity, suggesting that conductivity is high when the concentration of one type of base-pair is small. It is argued that increasing the disorderliness which leads to increase in the localization of the wave function causes a decrease in conductivity because more disorderliness results in decreasing the electron jump rate and consequently the conductivity. Moreover, for lower separation distance between guanine bases, $n < 3$ (n =number of AT base pairs between Guanine), the rate of charge transfer decreases with increasing separation as the charge transfer mechanism is dominated by tunneling. For larger separation between Guanine bases ($n > 3$), the transfer rates exhibit only weak distance dependence and hopping become dominant factor in charge transport mechanism.

Table 2.1: Experimental and Calculated Oxidation Potentials of Nucleotides [71].

Method	Solvent	dG	dA	dC	dT
Pulse Radiolysis	Aqueous	1.29	1.42	1.6	1.7
Electrochemistry	MeCN	1.49	1.96	2.14	2.11
Time-resolved Quenching	Aqueous	0.97	1.2		
DFT	Organic	1.88	2.01	2.18	2.25

Many studies have been conducted to investigate how DNA conductivity is affected by the nucleotide content [88][81][89][82][90][87][22][91][92][83]. A study by Yoo et al. [24] found that DNA conductivity varies significantly when the DNA molecule has different base pairs. Poly AT had approximately 100 times more resistivity than that of the poly GC. Some studies measured how conductive the short DNA molecules (8~32bp) are by using scanning tunneling microscopy. For example, Xu et al. showed that there is a linear relationship between the length of guanine and cytosine DNA wires and the conductivity. As the wire length increases, the conductivity decreases. If the wire is modified by attaching AT sequences on the GC wire, the conductivity decreases. Another study specifically showed that if the above modification happens in an 11bp, the conductivity drops by a factor of approximately 3. Another study found that GC DNA wires conduct better than AT DNA wires. Nogues et al. modified the DNA by changing GC content in the short DNA (26bp) using AFM. They found that the behavior of the varied GC content DNA was like that of the wide band-gap semiconductor. As the concentration of GC increases, the band-gap narrows. Moreover, Iqbal et al. studied the correlation between GC% and conductivity by

means of DC measurements in a dry environment. The short DNA molecules were bound through Au-thiol bonds between nano-gap gold bridges [32]. They showed that the DNA conductivity increases as GC% increases. Similarly, Dulic et al. showed that the DNA conductivity exponentially dropped as the AT base-pairs was inserted in GC DNA sequences. The results by Jortner et al. agreed to that of above researchers.

In sum, for micro-scale DNA wires, GC% content appears to play a significant role in the conductivity for both AC impedance and DC resistance with DNA wires containing a higher GC% displaying higher conductivity.

2.3.5. Long-Term Stability in DNA Molecular Wires

2.3.5.1 Effect of Temperature on Conductivity

Conductivity is a function of the concentration and mobility of free carriers available to conduct current[10], both of which are temperature dependent, with the mobility of charge carriers additionally influenced by the presence of charged impurities[24]. At lower temperatures, carriers move more slowly, and thus there is more time for them to interact with charged impurities. Consequently, as the temperature decreases, impurity scattering increases and the mobility of free carries decreases.

In general, a rise in temperature will typically stimulate two opposing phenomena in relation to electrical conductivity: external energy that excites electrons leading to an increase electrical conductivity via hopping, and lattice vibration and molecular expansion that reduces the electrical conductivity via tunneling[93]. In the case of organic semiconductors, electron-phonon

coupling and polaron formation complicate the picture. In highly disordered systems such as DNA, transport generally proceeds via hopping and is thermally activated.

Yu and Song modelled DNA as a 1D disordered system with electron transport occurring between localized states as variable range hopping which is highly temperature dependent[94]. In DNA, the dis-orderliness stems from random base pair sequence, resulting in localized electronic states that present the candidate landing sites for a hopping electron, while such localization would be improved by structural changes in DNA with temperature. There is a critical temperature above which the most likely hopping distance becomes smaller than the distance between bases, and above this temperature, the hopping mechanism can only be thermally activated. This explains the transition from weak temperature dependence to strong temperature dependence at around room temperature. Similarly, the work of Zhang and Ulloa, showing that transport property in DNA molecule is determined by not only disorder in base pair arrangement, but also by its correlation with hopping (at different temperature ranges), supports this theoretical model[95]. This explanation is also consistent with the study carried out by Bellido *et al.* which showed temperature dependences of the current–voltage characteristics of a triangular DNA origami deposited on a 100nm gap between platinum electrodes[96]. Tran *et al.* found similar behavior in that the conductivity is strongly temperature dependent around room temperature, with a crossover to a weakly temperature dependent conductivity at low temperatures[18]. The temperature dependence of conductivity has also been explained by the phonon-assisted tunneling mechanism proposed by Pipinys and Kiveris, where tunneling rate was considered to be a function of temperature[97]. Higher conductivity at higher temperatures was also reported by Kasumov *et al.*[55]. However, Iqbal *et al.* discovered a large decrease in conductivity at very high temperatures (400K) most likely due to the denaturing of the double-stranded DNA sequences. Further, Kassegne *et al.* have

shown that the temperature effects appear to be a two-staged, involving lower temperatures (4°C-40°C) and moderate to high temperatures (40°C-melting point). In the low temperature range, AC impedance increases until it reaches ~40°C. This is followed by a decrease in impedance until the melting point, resulting in the denaturation of λ -DNA strands and the loss of conductivity[76].

2.3.5.2. Effect of UV Irradiation

UV light is the most common source of DNA-molecular wire damaging radiation, and can potentially produce a variety of lesions in DNA in the form of (i) rupture of a strand, where both strands are broken at points less than 3 nucleotides apart, (ii) alteration to bases, in which bases are damaged, destroyed or chemically modified by radiation, and (iii) crosslinking and the formation of cyclobutane pyrimidine dimers (CPDs)[98]. CPDs are the most common and cytotoxic mutation which occurs between adjacent thymines on the same DNA strand. When the carbon- carbon double bonds of the pyrimidines are broken by UV light, four single-bonded carbon rings is formed which links the two thymines with CPDs. UV light could also form 6-4 phosphopproducts. This formation further results in the 44° kink [99]. On top of the DNA base damage, UV radiation also damages DNA phosphate groups. Gomes *et al.* reported AC conductivity of calf thymus DNA linked on gold electrodes exposed the sample UV-C light at preset time intervals with impedance measurements taken using alternating current from a range of 0.1Hz - 10 MHz [100]. They showed that the measured conductivity followed an exponential decay with respect to the time of irradiation. They suggested that the reduction in conductivity in DNA after UV light exposure occurred due to a decrease in the amount of phosphate groups that acted as an electron acceptor. Kuluncsics *et al.* reported that more lesions formed using less energy under UV-C light irradiation than any other light [101].The study by Jiang et al. also reported that as the dosage of UV light irradiation increased, the number of lesions in DNA increased [102].

Jiang et al. also used UV-C light-damaged supercoiled DNA to cut the damaged sites with T-4 endonuclease V. Then DNA was electrostatically immobilized by AFM to produce an image. The image showed that the sites with more fragments received more energy from the UV light, where more CPDs were formed. Thus, Temperature and UV light could affect the development of DNA wires. As a result, future work will need to have a controlled temperature and UV light environment to overcome this issue.

2.4. Conclusion

DNA self-assembly provide the most precise method to construct nanoscale objects of predefined size shape and compositions. The directly incorporated electronic/photonic functional properties allow connections and novel mechanisms to be formed within the organized structures. The combination of the properties allows ultimately for the creation of useful photonic and photovoltaic devices, DNA biosensors, and DNA diagnostic assay systems. Photonic properties of DNA are more understood compared to the electric properties as some groups showed the high efficiency of FRET in DNA construct. FRET has been used to measure the distance between the molecules which conjugated with fluorophores. Energy could transfer from one donor to one acceptor or from multiple donor to one acceptor. This nonradiative energy transfer could occur over more than 20 nm which includes more than 9 fluorophores. Different fluorophores are aligned from shortest to longest wavelengths of absorbance and emission. Major advantages of the FRET approach over electrical properties of DNA are: (i) extremely high sensitivity (1×10^{10} M strand concentration can be detected), (ii) excellent selectivity (detection of labeled strand in the presence of large excess of unlabeled oligonucleotide), and (iii) wide dynamic range of fluorescence measurements. These advantages support further development of DNA photonic transfer technology to yield viable application. DNA-based FRET probes have a wide variety of

applications in vivo and in vitro. FRET can be used to detect DNA hybridization. It can also be used as a biosensor to detect low concentration of DNA. FRET technique can also investigate the structural transitions of G-quadruplexes which are linked to potential diseases like cancer, HIV, and other diseases. However, it is hard to get the ideal form of the DNA-organized FRET-based photonic wires. The difficulties come from the photobleaching and dimerization of dyes.

The exact electrical properties of DNA are still unknown. Some groups have reported DNA to be an insulator, while others consider DNA to be a semiconductor and conductor. Regarding charge transfer mechanism, widespread experimental and theoretical studies over the past decade have presented different descriptions of charge transport mechanisms in DNA; there is significant disagreement on the mechanisms that rule over short distance, medium and long-range transport mechanisms in DNA. Moreover, different groups showed varied results on DNA conductivity. In some studies, short DNA has been found to have higher conductivity, and conductivity decreases by increasing the length of DNA. However, studies by another group have suggested the possibility of long-range transfer of electrons in DNA. These variable conclusions could be due to differences in environmental conditions of the experiments, and to differences in the instruments used to measure DNA conductivity. For example, dry DNA usually showed lower conductivity than wet DNA because there is an ionic current from water. Also, the substrate used often directly affects the conductivity of DNA and can mask the true conductivity of DNA molecular wires. Moreover, while some groups showed better conductivity in G4-DNA than the natural DNA, other groups showed better conductivity in DNA coated with conductive materials like metal ions. DNA molecular wire conductivity was not stable due to temperature and UV effect. Long-term stability has been a major concern in organic and molecular electronics where the stability is affected by such external factors like humidity, temperature and UV illumination.

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Chapter 3

Efficient Long-Range Florescent Energy Transfer in Multiple Donor-Single Acceptor Insulated DNA Nanostructures

3.1 Introduction

In the nature, antenna like structures that utilize FRET are found in the light harvesting photosystems of plants, algae and photosynthetic bacteria. In plants, the Photosystems I and II antenna are tightly packed membrane structures containing several hundred chlorophylls and accessory pigment molecules surrounding one photoreaction center chlorophyll [1][2][3][4][5]. In this process, the absorption of the photons excites the chlorophyll molecules and accessory pigments and raises the electrons to a higher energy level. The excited donor molecules are in effect an antenna which pass the energy to neighboring chlorophyll and other pigments molecules via the FRET process. The overall structures are arranged so that photonic transfer goes from higher energy (blue absorption) chlorophyll/pigment molecules to lower energy pigments (red absorption). All the photonic resonance energy collected by the antenna is efficiently transferred to a reaction center chlorophyll which called acceptor. The reaction center chlorophyll converts the photonic resonance energy to a charge separation, providing the energy and electrons for subsequent oxidations and reductions. These so-called “dark reactions” lead to water splitting, CO₂ fixation and all down-stream biochemical reactions. Because of the

overall high efficiency of the photon collection and FRET transfer, many attempts have been made to design artificial structures which mimic the antenna and other parts the photosynthetic systems [6][7][8][9][10]. Similar as the light harvesting complex in photosynthetic system, it should be possible to design DNA structures with a precisely spaced array of donor and acceptor 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[11][12][13][14].

In photonic antennas and wires, double-stranded DNA is used as a template to organize a linear array of fluorophores to accept a photon input at one terminus and emit a photon output at the opposite terminus. Excitonic energy is transferred nonradiatively along the length of photonic wires and between different fluorophores aligned from shortest to longest wavelengths of absorbance and emission. For example, early work by Kawahara et al. demonstrated energy transfer along a DNA wire with three different fluorophores over ~ 8 nm[5]. Ohya et al. and Heilemann et al., as seen in Figure 2.3, demonstrated energy transfer with five different fluorophores over distances of ~ 13 nm [6][7]. Spillman et al. did this with seven fluorophores over >16 nm [8]. Hannestad presented longer energy transfer over a distance of more than 20 nm [9]. As a result, studies showed that the energy could transfer between 1 donor to 9 donors.

Previous workers [13] have proposed for antenna or photonic amplification applications, the number of donors to acceptor could range from the lower limit of 2:1 to as high as 10^6 :1. When using heterogeneous DNA diagnostic and biosensor applications, the number of donors to an acceptor could range from the lower limit of 2:1 to upper limit of 10^5 :1. For homogeneous DNA diagnostic applications, the ratio of donors to acceptor could range from lower limit of 2:1 to upper limit of 10^4 :1.

Having a high FRET efficiency is critical in determining the photonic properties of DNA. This can be done by modifying the distance between multiples donors or between donors and acceptor. According to Heller et al. [20], the donor-donor inter-distance or the donor-acceptor inter-distance should be about 0.34 nm to 1.4 nm for FRET to be highly efficient. If the inter- distance is from 1.7 nm to 2.4 nm; the FRET was moderately efficient. If the inter-distance is larger than 2.8 nm, however, the FRET efficiency is very low. These considerations are crucial to the design of effective photonic DNA wires.

Despite knowledge of the optimal distance between DNA modifications, it is still hard to have the ideal conformation of the DNA-organized FRET-based photonic wires. Some of the difficulties come from the photobleaching and dimerization of dyes. Also, the dyes sometimes go through an undesirable direct excitation from their initial state. The dye dimerization is often common in the DNA with multiple donors [21][22][23][24][25]. However, in this study we showed that this can be resolved by adding surfactants and metal cations to the DNA.

3.2. Materials and Methods

All the sequences were supplied by Trilink Inc, San Diego, CA. For the donor 20mer ss-oligonucleotides (strand 1), three fluorescent TAMRA dyes (carboxytetramethyl-rhodamine) were attached to the thymine bases via C6-linkers (six methylene groups) with a 5-base spacing and 15mer dye -conjugated single-stranded (ss) oligonucleotides (strand 2) with 2 TAMRA dyes attached to thymine bases with a 5-base spacing. Strand 1 and 2 has been mixed to create 35 mer ss oligonucleotides, 5' GCA AC (dT-TAMRA) GCC TA (dT-TAMRA) AAT AT (dT-

TAMRA) TG 3' And 5' GAA (dT-TAMRA) ACG CC (dT-TAMRA) TCC GC 3'. For the acceptor 35 mer ss-oligonucleotides (strand 3,4 and 5), a single fluorescent Texas Red (sulforodamine) dye was attached via C6-linkers (six methylene groups) to the 6th adenine base from the 5'-terminal position or 6th adenine base from 3' terminal position or 18th adenine base from 5' terminal. 5' GCG GAA GGC GTA TTC CAA ATA TTA TAG GC (dA-C6-NH) (Texas-Red-X) GTT GC 3'. 5' GCG GAA GGC GTA TTC CA (dA-C6-NH) (Texas-Red-X) ATA TTA TAG GCA GTT GC 3'. 5' GCG GA (dA-C6-NH) (Texas-Red-X) GGC GTA TTC CAA ATA TTA TAG GCA GTT GC 3' are strands 3,4 and 5 respectively. For the control sequences, A complementary 35mer oligonucleotide without a Texas Red dye was also synthesized. A control complementary 20mer oligonucleotide without a TAMRA dye was synthesized. A control complementary 15mer oligonucleotide without a TAMRA dye was synthesized. The concentrations of the ss-DNA oligonucleotide solutions were determined by UV-Vis absorbance measurements. For preparing the hybridized double-stranded (ds) DNA structures, 10 μ M of the five TAMRA conjugated DNA (strand 1 and 2) was mixed with 10 μ M of the Texas Red conjugated complementary strand (strand 3, 4 and 5) or with the blank complementary strand (strand 6, 7, 8) in 0.5 \times PBS, then heated to 60 $^{\circ}$ C and cooled down slowly to room temperature 20 $^{\circ}$ C for 2 hours. Fluorescent emission and absorbance spectra of the hybridized ds-DNA FRET structures were obtained using Florescence spectrometer and UV-Vis. The standard excitation and emission values for the TAMRA donor dyes are (Ex. max 555 nm) and (Em. max 580 nm), and for the Texas Red dye are (Ex. max 595 nm) and (Em. max 615 nm) [25]. Table 3.1 shows the oligonucleotide sequences and positions of the donor and acceptor dyes, and Figure 3.1 shows simple schematic representations of all possible ds-DNA structures. All dye-conjugated DNAs were excited at 520 nm and emissions were measured

from 550 nm to 700 nm. While, for antenna effect calculations all dye conjugated DNAs were excited at 555 nm (excitation maximum wavelength of the donor) and 595 nm (excitation maximum wavelength of the acceptor). For FRET tests with multiple dyes present, to quantitatively extract two different emission intensities of the acceptor and donor from the overlapped spectra, pure TAMRA and TexasRed intensities were obtained by linear decomposition method. Specifically, the measured FRET signal was fitted by a linear function where $\text{FRET intensity} = a * \text{donor intensity} + b * \text{acceptor intensity}$. The coefficients a and b were empirically identified so that the model could best fit the measured signal.

In the multi-donor and single acceptor DNA systems, the emission intensity of the donors is highly influenced (quenched) by dimerization. The Fluorescence intensity could be compensated by adding surfactant

Table 3.1: DNA sequences and the positions of donor and acceptor dyes

DNA	Sequence
1	5' GCA AC (dT-TAMRA) GCC TA (dT-TAMRA) AAT AT (dT-TAMRA) TG 3'
2	5' GAA (dT-TAMRA) ACG CC (dT-TAMRA) TCC GC 3'
3	5' GCG GAA GGC GTA TTC CAA ATA TTA TAG GC (dA-C6-NH) (Texas-Red-X) GTT GC3'
4	5' GCG GAA GGC GTA TTC CA (dA-C6-NH) (Texas-Red-X) ATA TTA TAG GCA GTT GC 3'
5	5' GCG GA (dA-C6-NH) (Texas-Red-X) GGC GTA TTC CAA ATA TTA TAG GCA GTT GC 3'
6	5' GCA ACT GCC TAT AAT ATT TG 3'
7	5' GAA TAC GCC TTC CGC 3'
8	5' GCG GAA GGC GTA TTC CAA ATA TTA TAG GCA GTT GC 3'

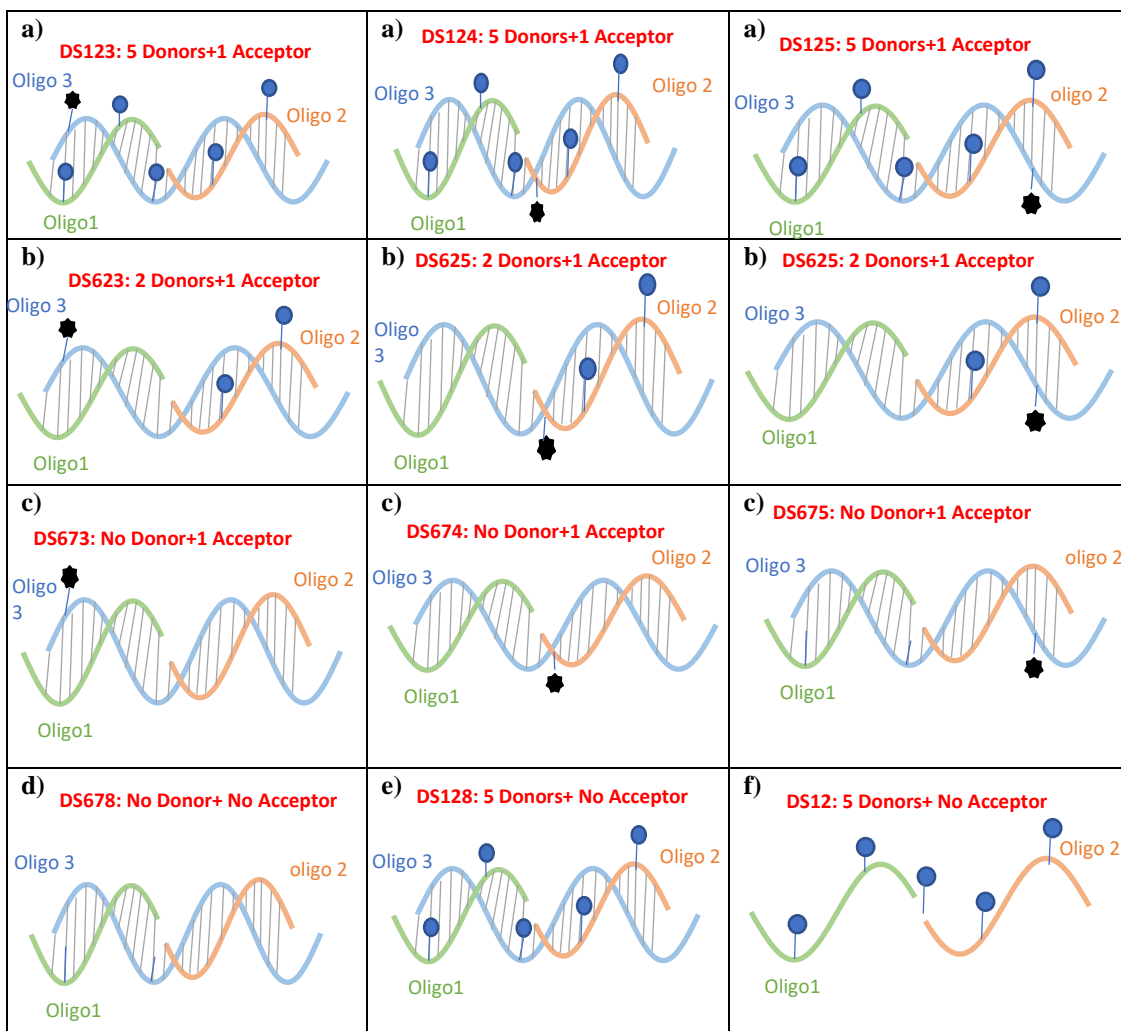


Figure 3.1: The schematics representation for: (a) the Five fluorescent TAMRA donor dyes on ds-DNA sequences with the presence of the fluorescent Texas Red acceptor dye in different location; and (b) the two fluorescent TAMRA donors in FRET ds-DNA hybrid structures with the fluorescent Texas Red acceptor dye present in the complementary sequence in different locations. (c) No TAMRA donors with the fluorescent Texas Red acceptor dye present in the complementary sequence (d) No TAMRA donor without The Texas Red acceptor. (e) the five fluorescent TAMRA donors in FRET ds-DNA hybrid structures without the fluorescent Texas Red acceptor dye (f) the five fluorescent TAMRA donors in ss-DNA structures without the fluorescent Texas Red acceptor dye.

and metal cations[20][26]. For this reason, Sodium dodecyl Sulfate (SDS) which is an anionic surfactant with addition of NaCl, MgCl₂ cations has been used. 10 mM SDS plus 50 mM Mg⁺² (the optimized concentration for the surfactant and metal cations [25]) has been added to the DNA systems. SDS with Mg⁺² cations appear to reduce the quenching, as shown in Figure 3.2. One hypothesis being that the Mg⁺² cations screen the repulsive forces between DNA backbone and SDS micelle negative charges, and now the dye-dye and other quenching are reduced by association with the hydrophobic areas in the micelle.

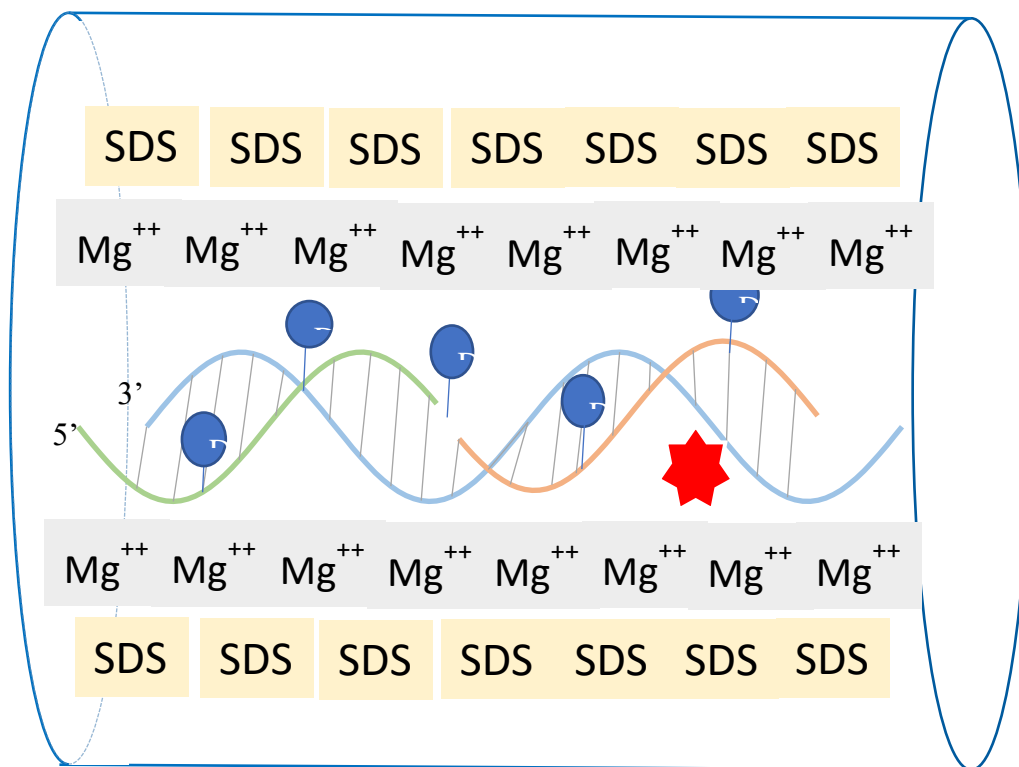


Figure 3.2: The sheathing effect of SDS and Mg⁺²

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. To quantify the FRET performance for the five 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 555nm, to the TexasRed acceptor emission when the acceptor alone is excited at 595 nm, as shown in Equation 1[25].

$$\text{Antenna Effect} = \frac{\text{Acceptor Emission}_{\text{multiple donors excited at 555nm}}}{\text{Acceptor Emission}_{\text{single acceptor excited at 595nm}}}$$

3.3 Results and Discussion

As shown in Figure 3.3, 3.4 and 3.5, the intensity of pure donor quenched by transferring energy to the acceptor and the acceptor increased fluorescence emission is observed. For ds-DNA, sequence 123 as shown in Figure 3.3, FRET efficiency based on the change of donor intensity is $(96-35)/96*100=64\%$ and based on ratio of intensity change of acceptor to donor is $(30-5)/96*100=26\%$. For ds124 as shown in Figure 3.4, FRET efficiency based on the change of donor intensity is $(96-32)/96*100=65\%$ and based on ratio of intensity change of acceptor to donor is $(35-4)/96*100=29\%$. For ds125 as shown in Figure 3.5, FRET efficiency based on the change of donor intensity $(96-29)/96*100=70\%$ and based on ratio of intensity change of acceptor to donor is $(28-5)/96*100=24\%$. This shows that for the long-range energy transfer, the position of the acceptors has a small effect on FRET efficiency.

Addition of negatively charged SDS does not influence fluorescence intensity; however, adding magnesium cations (Mg^{2+}) leads to much higher FRET efficiency as shown in Figure 3.6, 3.7 and 3.8. This improvement is likely due to the reduction of dimerization and other quenching effects due to the sheathing by SDS and Mg^{+2} as shown in Figure 3.2. This sheathing effect

probably breaks hydrophobic interactions between the dyes which causes quenching but allows them to reside in micellular environment which protects them from other quenching effects. For ds123, using SDS and Mg^{2+} , FRET efficiency based on donor intensity change increased from 64% to $(96-12)/96*100=88\%$. While the ratio of intensity changes of acceptor to donor increase from 26% to $(72-2)/96 *100= 73\%$ eff. For ds124, FRET efficiency based on donor intensity change increased from 65% to $(96-14)/96*100=85\%$. While the ratio of intensity changes of acceptor to donor increased from 29% to $(77-4)/96 *100= 76\%$. For ds125 as shown in Figure 3.5, FRET efficiency based on the change of donor intensity increased from 70% to $(96-6)/96*100=94\%$ and the ratio of intensity change of acceptor to donor increased from 24% to $(67-4)/ 96 *100= 65\%$.

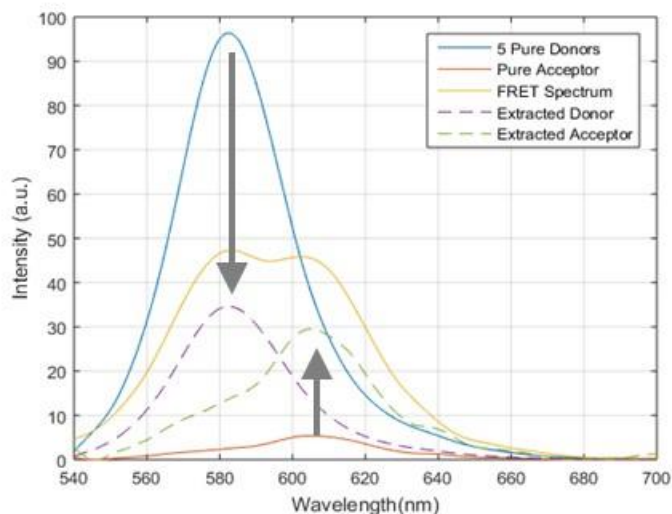


Figure 3.3: Fluorescence emission spectroscopy of ds123. Pure donor (ds128) (blue line), pure acceptor (ds673) (red line). FRET spectrum (orange line). Extracted donors as a dashed line and extracted acceptor as a dashed line.

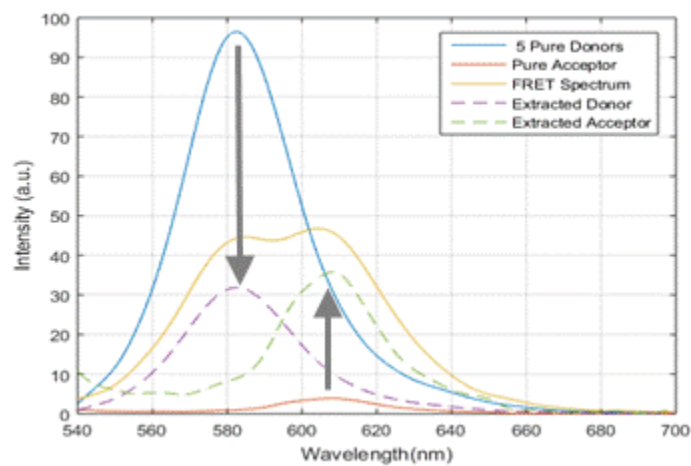


Figure 3.4: Fluorescence emission spectroscopy of ds124. Pure donor (blue line), pure acceptor (red line). FRET spectrum (orange line). Extracted donor as a dashed line and extracted acceptor as a dashed line.

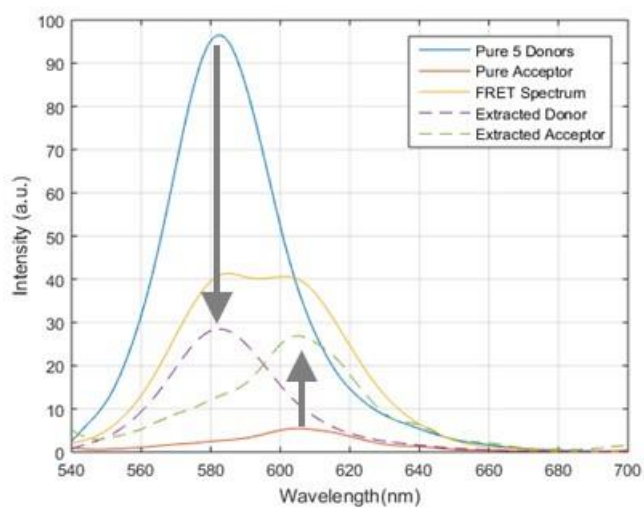


Figure 3.5: Fluorescence emission spectroscopy of ds125. Pure donor (blue line), pure acceptor (red line). FRET spectrum (orange line). Extracted donor as a dashed line and extracted acceptor as a dashed line.

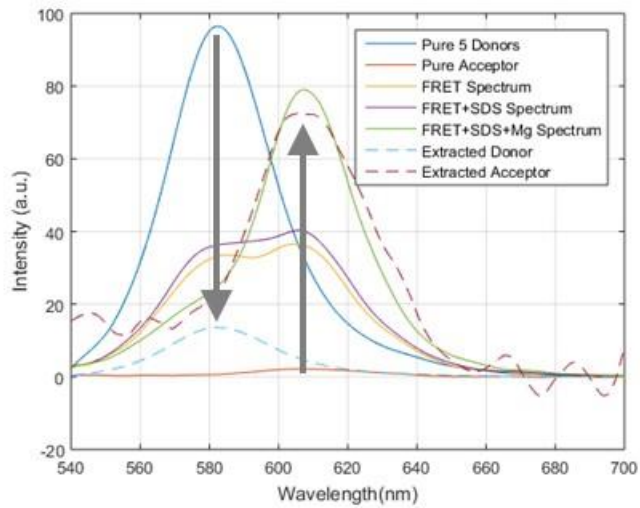


Figure 3.6: Fluorescence emission spectroscopy of ds123 when adding the surfactant and metal cations. Pure donor (blue line), pure acceptor (red line). FRET spectrum (orange line). FRET+SDS (purple line). FRET +SDS+ Mg^{+2} spectrum (green line). Extracted donor for FRET+SDS+ Mg^{+2} as a dashed line and extracted acceptor for FRET+SDS+ Mg^{+2} as a dashed line.

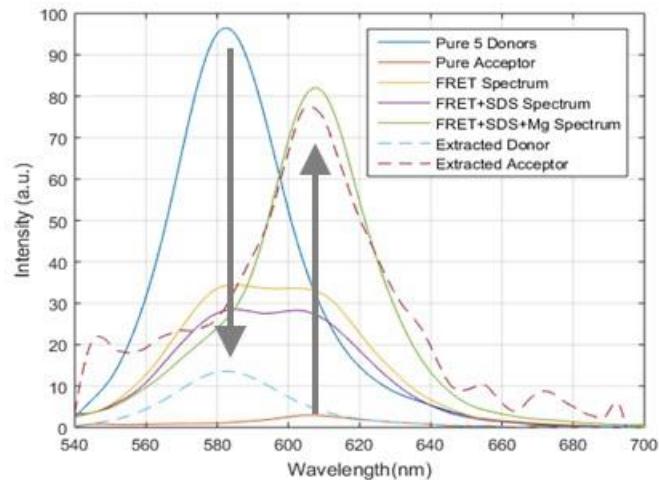


Figure 3.7: Fluorescence emission spectroscopy of ds124 when adding the surfactant and metal cations. Pure donor (blue line), pure acceptor (red line). FRET spectrum (orange line). FRET+SDS (purple line). FRET +SDS+ Mg^{+2} spectrum (green line). Extracted donor for FRET+SDS+ Mg^{+2} as a blue dashed line and extracted acceptor for FRET+SDS+ Mg^{+2} as a red dashed line.

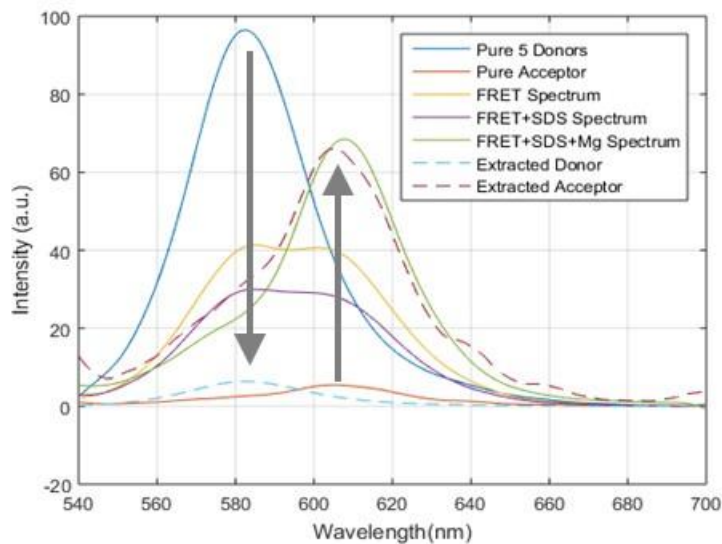


Figure 3.8: Fluorescence emission spectroscopy of ds125 when adding the surfactant and metal cations. Pure donor (blue line), pure acceptor (red line). FRET spectrum (orange line). FRET+SDS (purple line). FRET +SDS+ Mg^{+2} spectrum (green line). Extracted donor for FRET+SDS+ Mg^{+2} as a dashed line and extracted acceptor for FRET+SDS+ Mg^{+2} as a dashed line.

Figure 3.9 shows the FRET efficiency based on donor intensity reduction (white bar) for ds123, ds 124 and ds125 and based on intensity increase of acceptor (red bar), under pristine conditions. The addition of SDS and Mg^{+2} improves the FRET efficiency for all the DNA constructs. All the experiments are repeated 3 times. Bars indicates average values and error bars indicate standard deviation.

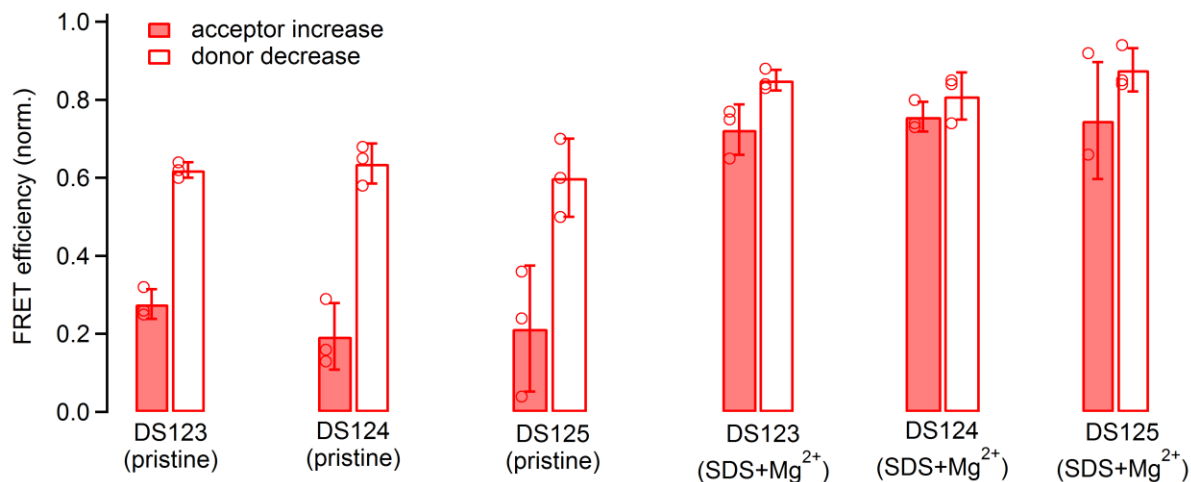


Figure 3.9: The FRET efficiency based on donor intensity reduction (white bar) for ds123, ds 124 and ds125 and based on intensity increase of acceptor (red bar), under pristine conditions. The addition of SDS and Mg²⁺ improves the FRET efficiency for all the DNA constructs. All the experiments are repeated 3 times. Bars indicates average values and error bars indicate standard deviation.

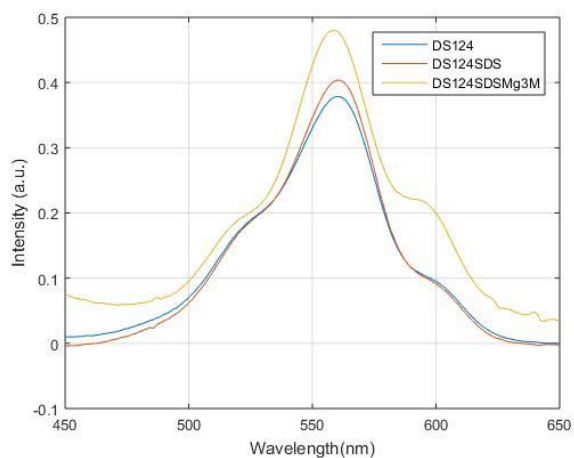


Figure 3.10. Absorbance intensity for ds124 (blue line), absorbance intensity for ds124 +SDS (red line) and the absorbance intensity for ds124 +SDS +Mg²⁺ (orange line).

Quantification of TAMRA's dimerization is comparing absorbance ratio of monomer (554nm) to dimer (520nm). As shown in Figure 3.10, higher absorbance ratio of monomer to dimer for ds124 of about 8% was observed when adding SDS and Mg²⁺ to the DNA solution

(ds124, $\frac{A_{554}}{A_{520}} = 0.38/0.15 = 2.5$ and for ds124+SDS+Mg²⁺, $\frac{A_{554}}{A_{520}} = 0.48/0.18 = 2.7$). This indicates somewhat less dimerization is observed with SDS and Mg²⁺.

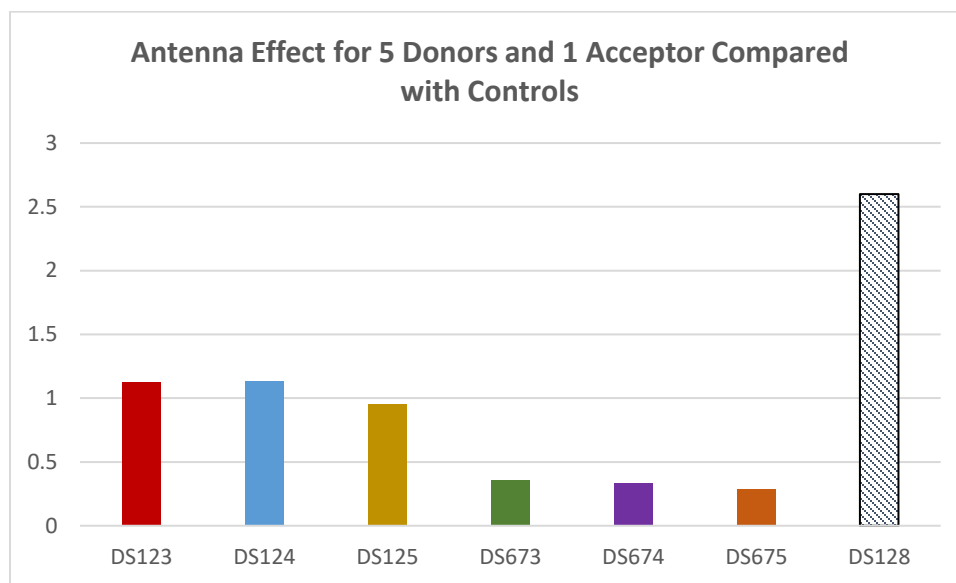


Figure 3.11: Antenna Effect for 5 donors and 1 acceptor (ds123, ds124, ds 125) compared with controls (ds673, ds674, ds675) and with 5 donors/no acceptor (ds128) under pristine conditions. The ds128 DNA with 5 donors and no acceptor shows the maximum expected antenna effect.

The Antenna Effects for ds 124, 123 and 125 under pristine conditions were calculated using equation 1 and were compared with the control sequences. For example, for ds123, ds 124 and ds125 the antenna effect are calculated as follow:

For ds123 $\frac{I_{A(Ex555)}}{I_{A(Ex595)}} = 70/62 = 1.13$, ds124 $\frac{I_{A(Ex555)}}{I_{A(Ex595)}} = 62/55 = 1.13$ and for ds125 $\frac{I_{A(Ex555)}}{I_{A(Ex595)}} = 105/110 = 0.95$.

An Antenna Effect (AE) value greater than one is an indication that the more distal donor groups in ds-DNA structure are contributing to the FRET emission of the single acceptor group. Under the most ideal FRET conditions (proximity, no quenching, etc.), a single donor to acceptor would have a value no greater than one.

We also studied the short-range energy transfer in DNA Nano construct where we have 2 donors and one acceptor in different location. For ds623 the distance between Texas red and the acceptor is 18 base pairs. For ds624 the distance between the Texas Red and the TAMRA is 6 base pairs, and for ds125 the acceptor is in front of the second TAMRA. For this arrangement, ds 623 has low FRET as the distance between the donor and the acceptor is 18 base pairs. For ds624 and ds 625, high FRET and medium FRET has been seen respectively as shown in Figure 3.12.

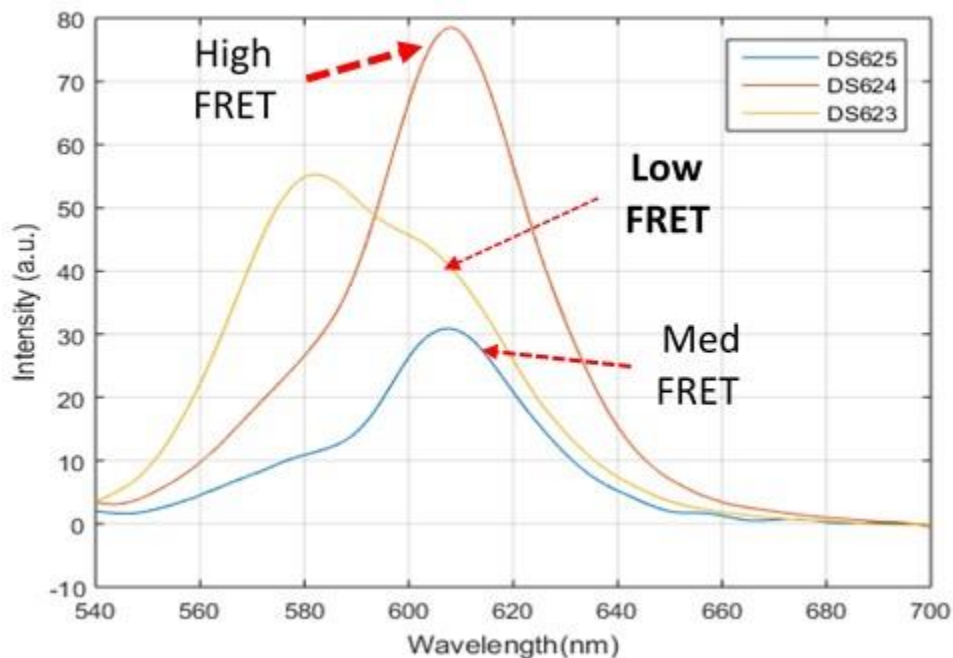


Figure 3.12: Short range energy transfer in DNA nanoconstruct.

3.4 Conclusion

For long-range FRET transfer, three 35 base pair DNA sequences (ds123, ds124 and ds125) with five donors and single acceptor which had 5 base pairs between the donors were designed, synthesized and tested for extended FRET transfer and the antenna effect. We observed that the fluorescent intensity of the donors in these ds-DNA structures were significantly reduced by transferring resonant energy to the acceptor, resulting in fluorescence re-emission by the acceptor. We also showed that the FRET efficiency of all three sequences ds123, ds124 and ds125 were similar, and concluded that the relative donor positions had little or no effect on overall FRET efficiency ($> 5\text{bp}$ or 1.7nm) for the five donor and single acceptor ds-DNA structures. However, for the short-range energy transfer where we had two donors and a single acceptor, the position of the donor relative to the acceptor had a more significant effect on the FRET efficiency.

Our most important result was showing that addition of SDS and Mg^{+2} significantly improved the FRET efficiency, with 70%-80% of the five TAMRA donor emission being transferred and re-emitted by the single Texas Red acceptor. Since three of the TAMRA donor groups are beyond an efficient Forster distance ($>12\text{bp}$ or 4.08nm), these results strongly support long range transfer and an antenna effect. We hypothesize that the addition of SDS and Mg^{+2} produce a sheathing or insulating effect that may be reducing fluorescent dye dimerization and the competing quenching mechanisms that greatly reduce the overall FRET efficiency under pristine conditions.

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Acknowledgment

Chapter 3 is the reprint of some material currently being prepared for submission as it appears in Golkar Narenji, A., Heller, M., Efficient long-range fluorescence energy transfer in multiple donor single acceptor insulated DNA Nano construction. The author is the primary researcher/author of this work.

Chapter 4

Brief Review on Effect of Temperature, UV Illumination and Nucleotide Content on the Electrical Characteristics of DNA

4.1 Introduction

Over the past years, a considerable amount of research has been conducted on the use of DNA as a potential molecular electrical wire [1-5]. The small size of DNA (diameter of 2nm) and its unique self-assembly and self-replicating properties make it a strong candidate for nanoelectronic devices [6]. Although the debate over the electrical properties and the exact charge transfer (CT) mechanism within DNA molecular wires continues, there is an increasing group of work supporting a semi-conductor behavior [7-16]. A relatively well-accepted model assumes a DNA molecule to behave as a one-dimensional aromatic crystal with π -electron conductivity [17].

The first approach investigating the use of DNA as a conductive material was published in the 1960's by Eley and Spivey, who reported DNA conductivity on the order of $10\text{-}12 \text{ }\Omega\text{cm}^{-1}$ and energy gaps (ΔE) of approximately $2.42 \pm 0.05\text{eV}$ at 400K [10]. Charge transfer mechanisms in DNA can be divided into two main transport models: short-range electron tunneling and charge hopping. Electron tunneling is a natural process in biology that occurs in all metabolic and energy transport processes [18]. Based on earlier studies on electron tunneling along DNA molecules, tunneling occurs through the π -stacking of the phosphate backbone [19], and is mainly dependent on the distant between donor and acceptor molecules. On the other hand, charge hopping involves the transport of electrons through base pairs, and exhibits only a weak dependence on the

separation distance between donor and acceptor. Moreover, the hopping mechanism becomes even more complicated as the ionization potentials for different DNA bases are not equal ($G < A < C, T$), see table 2.1; Guanine has an ionization potential of 7.85eV, Adenine 8.02eV, Cytosine 8.19eV, Thymine 8.46eV, AT base pairs 9.48eV and GC base pairs 7.34eV [20]. Because GC base pairs have a lower ionization potential, they are the preferred sites for charge hopping, and consequently it has been shown that, for DNA strands of similar length, a higher GC base pair content leads to an increase in conductivity [21].

The conductivity of DNA molecules depends on many factors including not only the structure and the surrounding chemical environment, but also the interaction with the substrate surface [22]. The research by Kassegne eliminated the effect of the substrate by attaching DNA molecular wires to high aspect-ratio three-dimensional (3D) microelectrodes [15]. Furthermore, they investigated the long-term performance of DNA molecular wires, particularly the electrical behavior in the presence of external effects such as UV irradiation and temperature. Because DNA is a large polymer made up of a long chain of nucleotides, it is expected that any damage to a nucleotide or a break in the chain will affect its electrical properties, and thus any external effects that can cause such damage in an expected and reproducible way can be used to gain a better understanding of charge transport mechanisms within DNA.

4.2. Effect of Temperature

The electrical conductivity of DNA molecular wires is directly affected by temperature. Conductivity is a function of the concentration and mobility of free carriers available to conduct current [2], both of which are temperature dependent, with the mobility of charge carriers

additionally influenced by the presence of charged impurities [23]. Impurity scattering is typically caused by crystal defects such as ionized impurities. At lower temperatures, carriers move more slowly, and thus there is more time for them to interact with charged impurities. Consequently, as the temperature decreases, impurity scattering increases and the mobility of free carries decreases.

In general, a rise in temperature will typically stimulate two opposing phenomena in relation to electrical conductivity: external energy that excites electrons leading to an increase electrical conductivity via hopping, and lattice vibration and molecular expansion that reduces the electrical conductivity via tunneling [24]. In the case of organic semiconductors, electron-phonon coupling and polaron formation complicate the picture. Moreover, in highly disordered systems such as DNA, transport generally proceeds via hopping and is thermally activated; disorder in a DNA system stems from the random base pair sequence, resulting in localized electronic states. These localized states present the candidate-landing site for the hopping electron, and such localizations are altered by structural changes in DNA that occur with changes in temperature. There is a critical temperature above which the most likely hopping distance becomes smaller than the distance between bases, and above this temperature, the hopping mechanism can only be thermally activated. This explains the transition from weak temperature dependence to strong temperature dependence at around room temperature. This explanation is consistent with the study carried out by Bellido et al which showed a temperature dependences of the current–voltage characteristics of a triangular DNA origami deposited on a 100 nm gap between platinum electrodes [25].

Tran et al showed a similar temperature dependency on the conductivity of DNA wires; the conductivity is strongly temperature dependent around room temperature with a crossover to a weakly temperature dependent conductivity at low temperatures [26]. Higher conductivity at

higher temperatures was also reported by Kasumov et al [27] whereas Iqbal et al [28] discovered a large decrease in conductivity at very high (400K) most likely due to the denaturing of the double-stranded DNA sequences.

Narenji, et al also shows the temperature dependency of DNA wires conductivity [29]. They used high aspect ratio chips to characterize the DNA. Their results show that temperature has a significant effect on the impedance of DNA molecular wires. As shown in (Figure 4.1a), the temperature effects appear to be a two-staged, involving lower temperatures (4°C-40°C) and moderate to high temperatures (40°C-melting point). In the low temperature range, AC impedance increases until it reaches ~40°C. This is followed by a decrease in impedance until the melting point, resulting in the denaturation of λ -DNA strands and the loss of conductivity. They also reported [15] subsequent renaturation of previously denatured λ -DNA molecular wires through incubation in a TM buffer solution at annealing temperature (900C) followed by temperature recycling (Figure 4.2b) resulted in the recovery of conductivity.

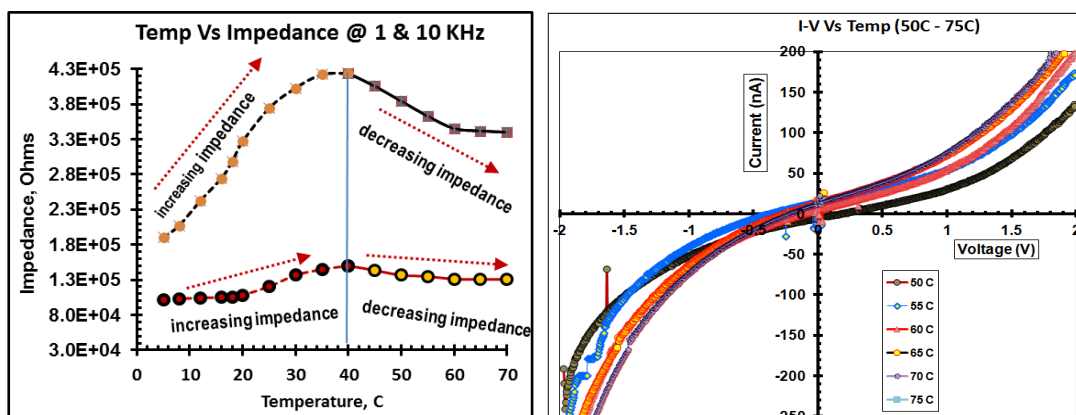


Figure 4.1: (a) Variation of AC impedance of λ -DNA molecular wire with temperature (0.5 ng/ μ L concentration). (b) Variation of I-V curve with temperature for λ -DNA molecular wire (0.125 ng/ μ L concentration)

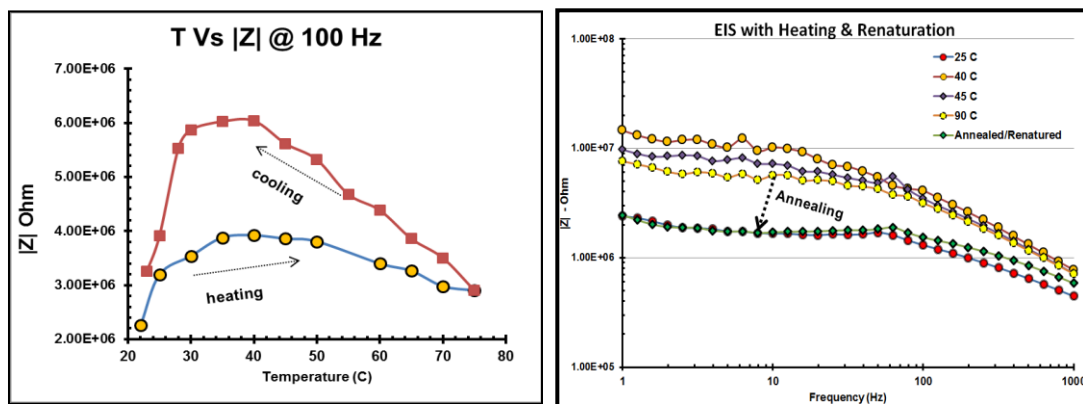


Figure 4.2: Temperature cycling of λ -DNA results in hysteretic behavior mainly due to partial denaturation (a) at 100 Hz, ($C_{DNA} = 0.125 \text{ ng}/\mu\text{L}$), (b) annealing (renaturation) in DNA Molecular wires.

4.3. Effect of UV Irradiation

UV light is the most common source of DNA-damaging radiation, and can potentially produce a variety of lesions in DNA in the form of (i) a rupture of a strand, where both strands are broken at points less than 3 nucleotides apart, (ii) alteration to bases, in which bases are damaged, destroyed or chemically modified by radiation, and (iii) crosslinking and the formation of cyclobutane pyrimidine dimers (CPDs) [30]. CPDs are the most common and cytotoxic biochemical mutation, occurring between adjacent thymines on the same strand of the DNA. Essentially, UV light energy excites and breaks the carbon-carbon double bonds in the pyrimidines and causes a four, single-bonded carbon ring to form; thus linking together the two thymines with CPDs forming a 7° - 9° bend. UV irradiation effects can also result in formation of 6-4 phosphoprotucts (6-4 PPs) forming a much more dramatic 44° kink [31]. UV light causes damage not only to base-pairs, but also phosphate groups reducing the path available both for hopping and short-range tunneling mechanisms, and hence increasing impedance. In this study the effect of temperature and UV irradiation on the electrical conductivity of DNA molecular wires was studied.

Narenji et al shows that the UV light exposure had a significant effect on the conductivity of the DNA molecular wires [29]. The amount of damage depended on the intensity, duration and wavelength of the UV light exposure (Figure 4.3). UV light irradiation caused the formation of pyrimidine dimers, opening of sugar rings and damage to phosphate group, which caused a loss of conductivity and thus an increasing impedance (Figure 4.3).

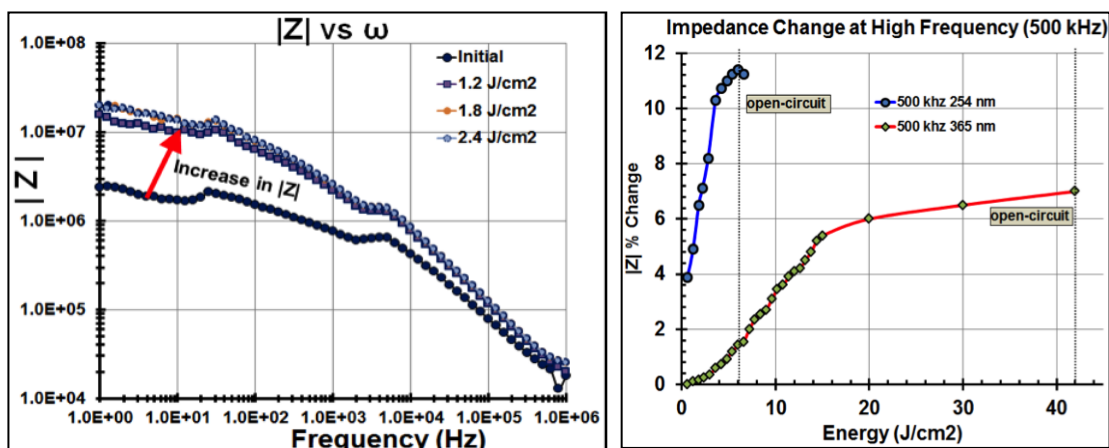


Figure 4.3: Change in impedance in DNA molecular wire subjected to UV irradiation. (a) Increase in Impedance while applying UV-C (254nm Wavelength) irradiation with different intensity (1.2-2.4J/cm²). (b) DNA subjected to both UV-A (365nm Wavelength) and UV-C irradiation.

4.4 Effect of Nucleotide Content

An increasing amount of research studies point towards the sequence dependency of DNA conductivity [32][33][34][35][36][37][38][39]. It has been reported that guanine has lower potential compare to other bases; therefore, it is the first candidate to hop. Consequently, triple G has a lower ionization potential as it has more overlapping between its π -orbital compared to single or double G base-pair; Ratner and co-workers reported that the ionization potentials of GG and

GGG were lower than G by 0.5 and 0.7eV, respectively[40]. Work by Saito and colleagues established that the GGG sequence was easier to oxidize than G[39].

The sequence dependence of conductivity was demonstrated by measuring the charge transfer mechanism with regard to localization length[36]. The localization lengths of poly (dG) – poly (dC) DNA tend to infinity, meaning that the sequence is perfectly conducting compare to Lambda DNA with 45% GC. Similarly, Song *et al.* showed that the rate of charge transfer depends on the distance and sequence context of the DNA[37]. However, the work also showed that randomness may play a bigger role in DNA conductivity, suggesting that conductivity is high when the concentration of one type of base-pair is small. It is argued that increasing the disorderliness which leads to increase in the localization of the wave function causes a decrease in conductivity because more disorderliness results in decreasing the electron jump rate and consequently the conductivity. Moreover, for lower separation distance between guanine bases, $<1n<3$ (n =number of AT base pairs between Guanine), the rate of charge transfer decreases with increasing separation as the charge transfer mechanism is dominated by tunneling. For larger separation between Guanine bases ($n>3$), the transfer rates exhibit only weak distance dependence and hopping become dominant factor in charge transport mechanism.

Many studies have been conducted to investigate how DNA conductivity is affected by the nucleotide content [32][42][33][34][38][43][44][45][46][47]. A study by Yoo et al. [43] found that DNA conductivity varies significantly when the DNA molecule has different base pairs. Poly AT had approximately 100 times more resistivity than that of the poly GC. Some studies measured how conductive the short DNA molecules (8~32bp) are by using scanning tunneling microscopy. For example, Xu et al. showed that there is a linear relationship between the length of guanine and

cytosine DNA wires and the conductivity. As the wire length increases, the conductivity decreases. If the wire is modified by attaching AT sequences on the GC wire, the conductivity decreases. Another study specifically showed that if the above modification happens in an 11bp, the conductivity drops by a factor of approximately 3. Another study found that GC DNA wires conduct better than AT DNA wires. Nogues et al. modified the DNA by changing GC content in the short DNA (26bp) using AFM. They found that the behavior of the varied GC content DNA was similar to that of the wide band-gap semiconductor. As the concentration of GC increases, the band-gap narrows. Moreover, Iqbal et al. studied the correlation between GC% and conductivity by means of DC measurements in a dry environment. The short DNA molecules were bound through Au-thiol bonds between nano-gap gold bridges [48]. They showed that the DNA conductivity increases as GC% increases. Similarly, Dulic et al. showed that the DNA conductivity exponentially dropped as the AT base-pairs was inserted in GC DNA sequences. The results by Jortner et al. agreed to that of above researchers.

Goshi et al, reported the results of the effects of nucleotide content on the conductivity of plasmid-length DNA molecular wires suspended between 3D microelectrodes [49]. Both AC and DC measurements methods to compare the conductivity of DNA plasmid wires was used. The DNA wires made with 63% GC (pMyC) content showed nearly twice the conductivity of DNA wires containing 39% GC (pCas 9) as shown in Figure 4.4. Although the humidity and temperature-controlled environment was used to store the chips, over time, the DNA molecular wire will lose its conductivity. Figure 4.5 Compares Impedance spectroscopy of pMyC and pCas9 DNA wires over period of 80 days. As seen in Figure 4.5, the overall impedance of pCas 9 is higher than pMyC during 80 days of experiment.

In summary, for micro-scale DNA wires, GC% content appears to play a significant role in the conductivity for both AC impedance and DC resistance. The DNA wires containing a higher GC% displaying higher conductivity.

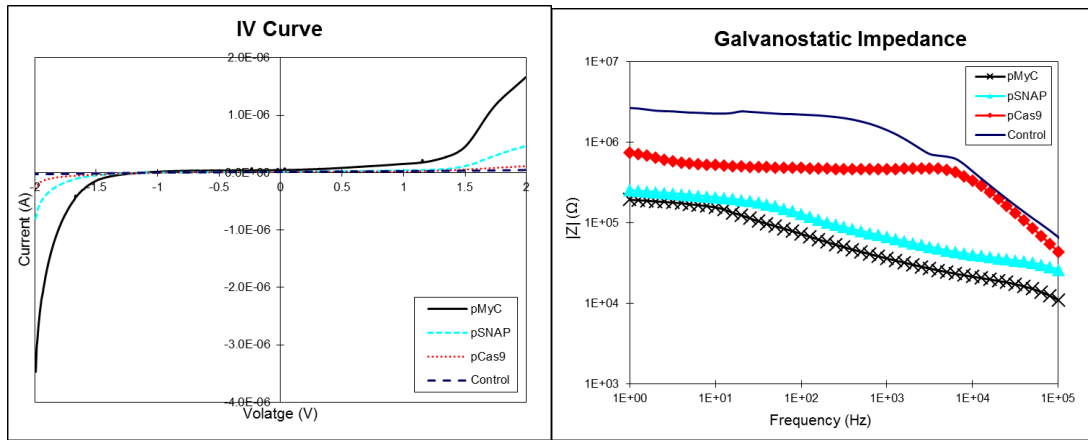


Figure. 4.4: Impedance spectroscopy and IV Curve comparing pMyC, pSNAP, and pCas9 DNA wires

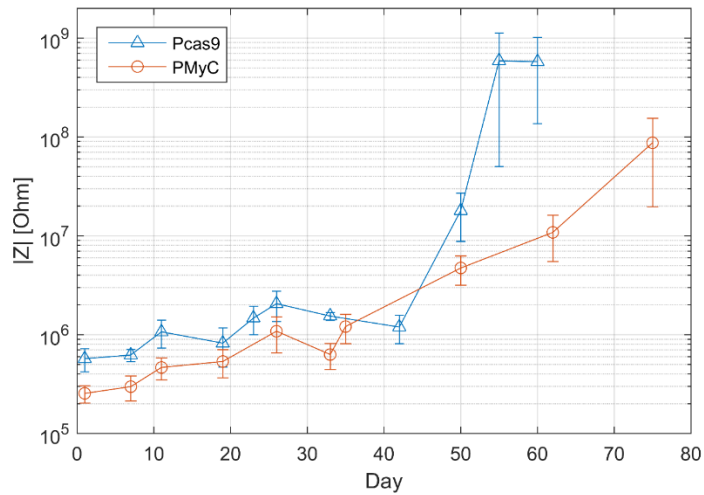


Figure. 4.5: Comparing Impedance spectroscopy of pMyC and pCas9 DNA wires over period of 80 days

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Chapter 5

Conclusion and Future Work

5.1. Conclusion

DNA self-assembly provide the most precise method to construct nanoscale objects of predefined size shape and compositions. The directly incorporated electronic/photonic functional properties allow connections and novel mechanisms to be formed within the organized structures. The combination of the properties allows ultimately for the creation of useful photonic and photovoltaic devices, DNA biosensors, and DNA diagnostic assay systems. Photonic properties of DNA are more understood compared to the electric properties as some groups showed the high efficiency of FRET in DNA construct. FRET has been used to measure the distance between the molecules which conjugated with fluorophores. Energy could transfer from one donor to one acceptor or from multiple donor to one acceptor. This nonradiative energy transfer could occur over a distance of more than 20 nm which includes more than 9 fluorophores. Different fluorophores are aligned from shortest to longest wavelengths of absorbance and emission.

Major advantages of the FRET approach over electrical properties of DNA are: (i) extremely high sensitivity (1×10^{10} M strand concentration can be detected), (ii) excellent selectivity (detection of labeled strand in the presence of large excess of unlabeled oligonucleotide), and (iii) wide dynamic range of fluorescence measurements. These advantages support further development of DNA photonic transfer technology to yield viable application. DNA-based FRET probes have a wide variety of applications in vivo and in vitro. FRET can be used to detect DNA hybridization. It can also be used as a biosensor to detect low concentration of DNA. FRET technique can also investigate the structural transitions of G-quadruplexes which are linked to potential diseases like cancer, HIV, and other diseases. However, it is hard to get the ideal form of the DNA-organized FRET-based photonic wires. The difficulties come from the photobleaching and dimerization of dyes. In this thesis, the 35 base pairs DNA sequences with 5 donors and 1 acceptor which has the distance of 5 base pairs between the donors has been designed. We observed that the intensity of pure donor quenched by transferring energy to the acceptor and the acceptor intensity increased. The average FRET efficiency of the ds-systems where the distance of the distal donor dyes to the acceptor dye becomes greater than 1.7 nm or five base pairs (which is beyond the optimal FRET distance) based on the change of donor intensity is 66%. However, we would be able to increase the fluorescence intensity to almost 90% by adding surfactant and metal cations. We showed that the addition of negatively charged SDS surfactant does not influence the fluorescence intensity; nevertheless, addition of magnesium cations (Mg^{2+}) to SDS lead to higher FRET efficiency.

The exact electrical properties of DNA are still unknown. Some groups have reported DNA to be an insulator, while others consider DNA to be a semiconductor and conductor. Regarding charge transfer mechanism, widespread experimental and theoretical studies over the past decade

have presented different descriptions of charge transport mechanisms in DNA; there is significant disagreement on the mechanisms that rule over short distance, medium and long-range transport mechanisms in DNA. Moreover, different groups showed varied results on DNA conductivity. In some studies, short DNA has been found to have higher conductivity, and conductivity decreases by increasing the length of DNA. However, studies by another group have suggested the possibility of long-range transfer of electrons in DNA. These variable conclusions could be due to differences in DNA sequences, environmental conditions of the experiments, and the instruments used to measure DNA conductivity. For example, dry DNA usually showed lower conductivity than wet DNA because there is an ionic current from water. Also, the substrate used often directly affects the conductivity of DNA and can mask the true conductivity of DNA molecular wires. In this thesis, we also reviewed the effect of temperature, UV and nucleotide content on electrical characteristic of DNA. In almost all the studies the sequences with higher GC content which has lower ionization potential have higher conductivity. Therefore, the conductivity of DNA wires can be tuned through varying the GC% content. Their results also suggest that charge transfer theories developed from experiments on nano-scale (<50bp) DNA wires, in particular the charge hopping mechanism, are still applicable at the micro-scale. Moreover, while some groups showed better conductivity in G4-DNA than the natural DNA, other groups showed better conductivity in DNA coated with conductive materials like metal ions. DNA molecular wire conductivity was not stable due to temperature and UV effect. Long-term stability has been a major concern in organic and molecular electronics where the stability is affected by such external factors like humidity, temperature and UV illumination. To prove this, in this thesis, the brief review has been done on effect of temperature and UV irradiation on the electrical conductivity of DNA molecular wires.

5.2 Future Directions

5.2.1 Future Work for Photonic Transfer in DNA nanoconstruct

Future FRET application is in the DNA circuit. The FRET between double-stranded DNA is used as the platform for dye-based photonic wires. Since the DNA nano structures are stronger than double-stranded DNA, they are used as building blocks of long photonic wires. Synergy of bottom-up self-assembly and top-down photolithography methods are used for organization of DNA Photonic and Electronic Transfer Structures in both 2D and 3D fashion which has been shown in Figure 5.1. The DNA Nano construct allows the multiple dimension construction (2D and 3D) and provides the various pathways for photonic and electronic transfer.

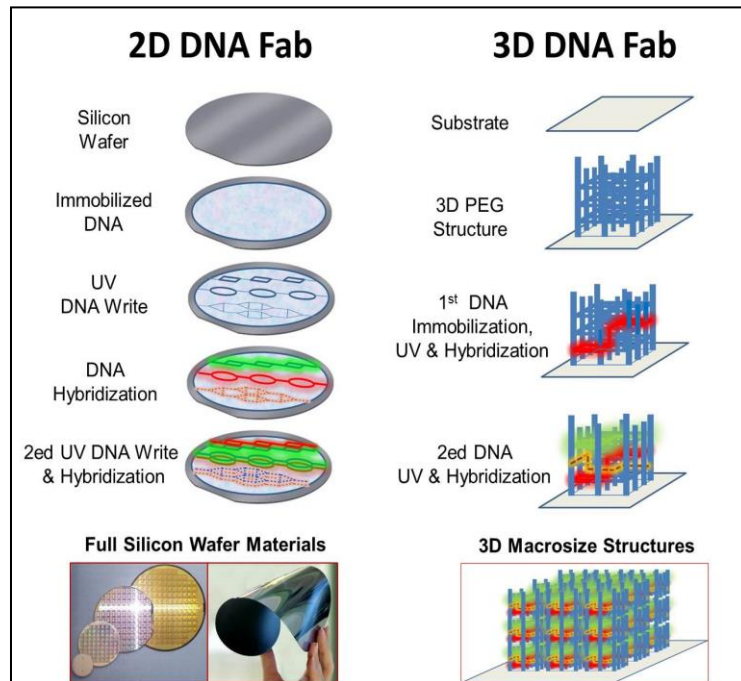


Figure 5.1: DNA Photonic and Electronic Transfer Structures and Devices Using 2D and 3D DNA Fabrication.

5.2.2 Future Work for Electronic Transfer in DNA Nanoconstruct

As a viable solution to long-term stability problems associated with DNA molecular wires along with the additional benefits of tuning their conductivity, hybrid DNA molecular wires made of natural base-pairs and synthetic base-pairs, which have lower ionization potentials and a more π orbital overlapping, offer an attractive option. A strong synthetic nucleotide candidate is tricyclic cytosine analogue (such as (8-MeO)tC), which we show has (i) a lower ionization potential ($\sim 0.65\text{V}$) compared to natural nucleotides, G (1.29V), C (1.6V), A (1.42V), T (1.7V) and (ii) more π -orbital stacking due to its tricyclic structure.

Figure 5.2 shows cyclic voltammetry analysis of (8-MeO) tC with different scan rates of 0.1, 0.2, 0.5, 1 and 2 V/s. As can be seen in the Figure, ionization potential of (8-MeO) tC was measured to be $\sim 0.65\text{V}$ (corresponds to oxidation peak), which is much lower than the natural bases, G (1.29V), C (1.6V), A (1.42V), T (1.7V) as shown in Figure 5.3.

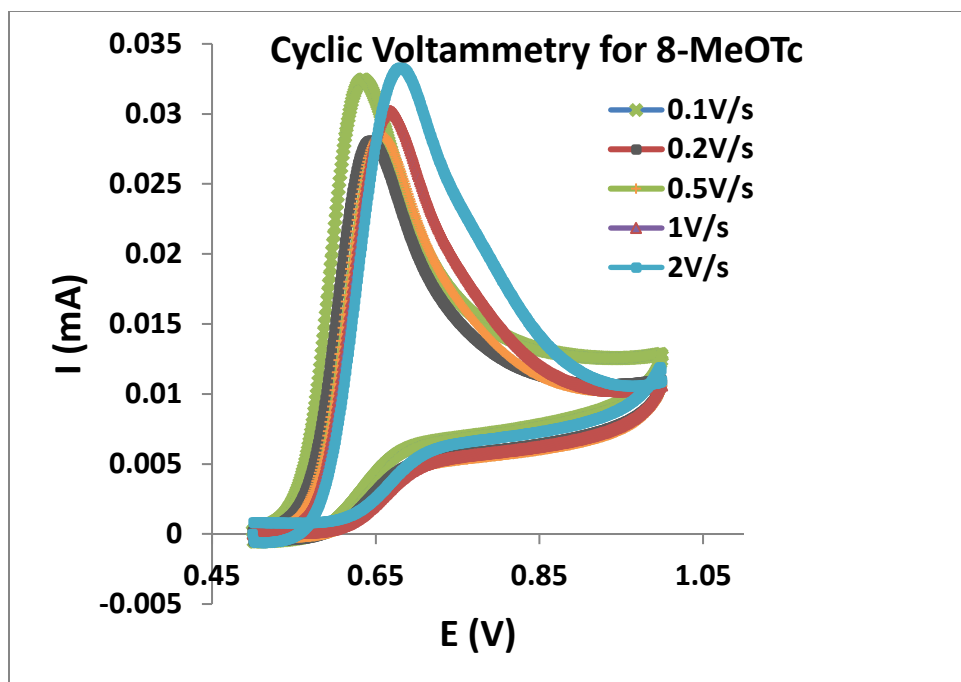


Figure 5.2: Cyclic Voltammetry of tricyclic nucleotides, 8-MeOTc under a variety of scan rates. The oxidation peak corresponds to ionization potential.

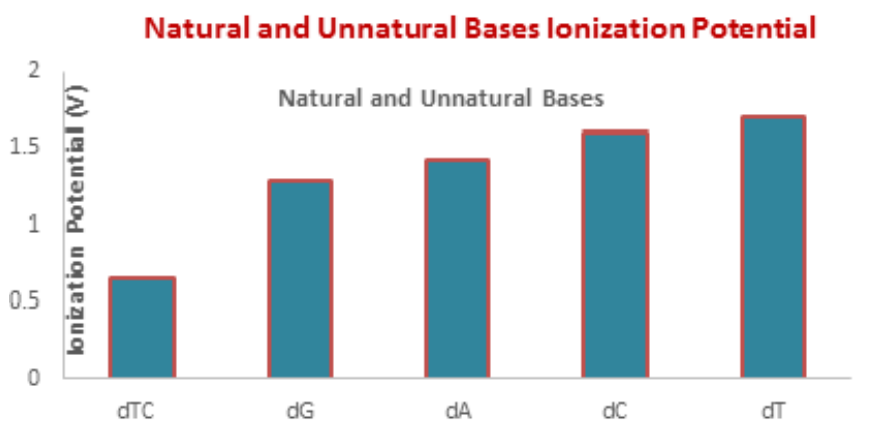


Figure 5.3: Comparing Natural and Unnatural Bases Ionization Potential.