DNA MOLECULAR WIRE-BASED NANOELECTRONICS: NEW INSIGHT AND HIGH FREQUENCY AC ELECTRICAL CHARACTERIZATION

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DEDICATION

I would like to dedicate this thesis to my family, my wife Ellinda Leonita, my son Alden Wibowo, and my daughter Bianca Wibowo, who always encourage and support me every single day. You are the reason behind all the greatness in my life.

I would also like to dedicate this to San Diego State University who given me a second chance, Dr. Sam Kassegne for the incredible support during my study, and all the members of MEMS Lab. who have been my extended family and made me never walk alone.
ABSTRACT OF THE THESIS

DNA Molecular Wire-Based Nanoelectronics: New Insight and High Frequency AC Electrical Characterization

by

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While recent research in electron-transport mechanism on a double strands DNA seems to converge into a consensus, experiments in direct electrical measurements on a long DNA molecules still lead to a conflicting result. This research investigates experimentally the attachment of DNA molecular wire to high aspect ratio three-dimensional (3D) metal electrode and the effect of temperature to its AC electrical conductivity. The 3-D microelectrode was built on a silicone oxide substrate using patterned thick layers of negative tone photoresist covered by sputtered gold on the top surface. Attachment of λ-DNA to the microelectrode was demonstrated using oligonucleotide-DNA phosphate backbone ligation and thiol-gold covalent bonding. Electrical characterizations based on I-V and AC impedance analysis of several repeatable data points of attachment with varying λ-DNA concentration (500 ng/µL to 0.0625 ng/µL) showed measurable and significant conductivity of λ-DNA molecular wires. Further study was carried out by measuring I-V and impedance while ramping up the temperature to reach complete denaturation (~110°C) resulting in no current transduction. Subsequent re-annealing of the DNA through incubation in TM buffer at annealing temperature (~90°C) resulted in recovery of electrical conduction, providing a strong proof that DNA molecular wire is the one generate the electrical conductivity. λ-DNA molecular wires reported to have differing impedance response at two temperature regions: impedance increases (conductivity decrease) between 4°C – 40°C, and then decreases from 40°C until DNA completely denatured (~110°C). The increase conductivity after 40°C is an experimental support the long distance electron transport mechanism referred as “thermal hopping” mechanism. We believe that this research represents a significant departure from previous studies and makes unique contributions through (i) modification of DNA attachment methods has increase the success rate from less than 10% to be more than 75%
(ii) more accurate direct conductivity measurement of DNA molecular wires facilitated by suspension of the DNA away from the substrate, and (iii) AC impedance measurement of DNA molecular wires with the effect of temperature suggests an experimental evidence of temperature gating mechanism in charge transport through DNA wire that will be very important for further studies.
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CHAPTER 1

INTRODUCTION

The ever increasing demand for more powerful computing devices continues to be a driving force in extending the miniaturization of semiconductors, the building block of high performance electronics systems [1]. However, this continuous demand of miniaturization has presented fundamental technology challenge, particularly for applications requiring mass-scalability. It is expected that as semiconductor dimensions approach the 5–7 nm range, it will be difficult to fabricate with lithography process. As we look ahead the years 2020–2025, we can see that many physical dimensions are expected to be crossing the 10 nm threshold [2]. Therefore, it is becoming clear that fundamental geometrical limits will be reached in the above timeframe and this situation has triggered the search for an alternative.

DNA molecular wire has been proposed as potential solution to this challenge and they continue to attract significant research attention and focus [3-7]. Traditionally, such research had centered on DC conductivity (whether DNA is conductive or not); but there is evidence of increasing interest in understanding DNA electrical behavior under AC signal. These research works are predominantly in the theoretical approach realm with focus on a 1-dimensional conduction model with far and between experimental works reported [8-9]. Theoretical approaches have concentrated on current transduction in DNA based on 1-dimensional disordered systems analogy where frequency-dependent charge hopping between localized states is assumed to this extremely be dominant [10-12]. This extremely limited experimental support for these theories suggests the need for comprehensive experimental work in AC. This thesis investigates experimentally the attachment of DNA molecular wire to high aspect ratio three-dimensional (3D) metal electrodes and subsequently high frequency AC electrical characterization as well as the temperature effect in such wires. Further, these efforts are used to test some of the leading theories of DNA charge transport mechanism.
1.1 Revolution of Semiconductors

The rapid growth of modern electronics starts with semiconductors, as a type of materials that can be induced to either conduct or resist the flow of electricity. What distinguishes electrical conductivity of semiconductors from conductors and insulators is basically what defines semiconductors as a separate class of materials. In contrast to conductors and insulators, electrical conductivity of semiconductors can be controlled by orders of magnitude by introduction of alien elements (doping) [13]. Furthermore, conductivity of semiconductors can be controlled by two types of carrier: negative electron and positive holes. In addition, its electrical conductivity also depends on temperature, illumination, as well as electric and magnetic fields [13].

Silicon is arguably the most important semiconductor that played an undeniably pivotal role in the explosive growth of electronics industry over the last six decades. Its use continues to grow not only through the needs of ever progressing microprocessor and memory integrated circuit technology, but also through the growing needs of solar cells (photovoltaic) and Micro-Electro-Mechanical Systems (MEMS) application [14]. The incredible growth of electronics industry was made possible by the remarkable technological progress coming from the transistor’s size reduction [15]. The industry’s history of steady increases in complexity was noted by Gordon Moore with the famous Moore’s Law which states that, ‘the complexity of an integrated circuit, with respect to minimum component cost, will double in about 18 months’ [16]. Figure 1.1 shows the implication of the Moore’s Law to the microprocessor industry, showing that the number of transistor inside a processor is double every year [15].

Over time that law has held up pretty well but in the end, exponential growth cannot continue forever [17]. The main problem is that processors are built with silicon. As silicon transistors get more and more dense, they need more power and better cooling. In other words, it is not that we cannot design faster chips, it is just too expensive and difficult to keep them running. This situation has leads the initiative to find non silicon material which can possibly prolong the life of Moore’s Law. The field that traditionally has been limited to silicon as demonstrated by the multi-billion dollar industry now understandably evolving into
a generic search for the best fabrication techniques involving non-silicon materials, witnessing the new era of molecular electronics [18].

![Figure 1.1. Moore’s Law reflecting the reduction in transistor size [15].](image)

The concept of molecular electronics is very much different from conventional solid state semiconductor electronics. It allows chemical engineering of organic molecules with their physical and electronic properties tailored by synthetic methods, bringing a new dimension in design flexibility that does not exist in typical inorganic electronic materials. The most common types of molecular wires are based on organic molecules. A molecular wire occurring in nature is DNA, and studies on a long-range conductivity in DNA wires have been quite intense.

### 1.2. BIO-INSPIRED NANOELECTRONICS

The field of nanoelectronics aims to enable the continued realization of Moore’s Law by using new methods and materials to build electronic devices with feature sizes on the nanoscale. Transistors will eventually reach the limits of miniaturization at atomic levels.
which is a fundamental barrier. New nanoscale electronic devices could mean new materials, new forms of integration, and/or new methods of fabrication/ manufacture. Exploration to find new materials based on self-assembly synthetic, and bio-inspired, materials is continuous.

Molecular and cluster beam epitaxial has been recognized as the early techniques of a nanofabrication method. More recently, the capability for defining lateral dimensions with an accuracy of nanometers has been achieved by the chemistry of self-assembly and molecular design. On the other hand, advanced lithographic and replication methods have provided an alternative in nanofabrication. These revolutionary techniques have facilitated the fabrication of structures with atomic layer resolution, and taken microelectronics into the nanoelectronics regime and serve the basic workhouse structures for the study of many novel quantum phenomena and device concepts [9].

There is always a search for reliable and environmentally friendly processes to manufacture nanomaterials and minimizing or even eliminating the use of hazardous chemicals. The only way to develop these sustainable processes is to adapt benign synthesis approaches that use mild reaction conditions and non-toxic reaction precursors [19]. Based on that purpose, the use of microorganisms to synthesize functional nanomaterials has been of great interest. One of the promising areas of modern research is the utilization of the inherent properties of nucleic acids like DNA to create useful materials. Today, with new and emerging chemical processes, bio-inspired nanoelectronics have the potential to provide a unique spectrum of new concepts and capabilities for future generations of electronics.

1.3. MOTIVATION OF STUDY

DNA is the blueprint for life. The possibility of long-range electron transfer through a DNA molecule has intrigued many researchers since a long time [20]. The structure of DNA, with its π-electron system of base pairs stacked in the core through the length of the molecule, is reminiscent of an ‘electric wire’. The questions whether DNA is a molecular wire and can be used for transferring electrons through its π-stacks are being investigated through various approaches [21]. While many other groups have worked on DNA wires and DNA immobilization on various surfaces, none of these groups have investigated how to work on a platform that allows for testing and development of DNA as a biosensor or an
electrical component [22-23]. The purpose of this study is to give a bigger picture of DNA electrical properties of DNA based molecular wire as the AC electrical characterization has been done in a wide range of frequency. Furthermore the study on the effect of temperature will also give a new insight on the temperature dependency of DNA based molecular wire in comparison to traditional solid materials and support the leading theories in DNA charge transport mechanism.

1.4. ORGANIZATION OF THESIS

In this study, Chapter 1 covers the Introduction while the summary of previous studies that has been done so far in the field of bionanoelectronics is provided in Chapter 2. Chapter 3 covers the properties of DNA as molecular wire from the biology, chemistry and biophysics point of view. Chapter 4 presents in detail the bionanoelectronic platform that includes information on the fabrication of the electrodes and microfluidics channel, methods of attachment, and the attachment optimization. In chapter 5, we present the result of electrical characterization including interesting founding in temperature effect, hysteresis of dsDNA, and re-annealing. Comparative discussion is given in Chapter 6 while the conclusion and future study are covered in Chapter 7.
CHAPTER 2

DNA-BASED BIONANOELECTRONICS

LITERATURE SURVEY

Over the past several years, a significant amount of research interest has focused on self-assembled systems through exploiting the molecular behavior of various natural biological materials like DNA [3-10]. Seeman et al. had investigated construction of structures using DNA molecules as scaffolds, which may ultimately serve as frameworks for nanoelectronics devices [11-12]. According to the model that seems to have gained more acceptance than competing theories, the promise of DNA as conducting molecular wire origins from the array of π-stacked base pairs that resemble conductive one-dimensional aromatic crystals [9].

Figure 2.1. Basic structural element of DNA. The blue line shows the phosphate backbone [20].

Along these lines, Mirkin et al. [9] and Alivisatos et al. [24] were the first to describe self-assembly of gold nanoclusters into periodic structures using DNA. Mirkin et al. described a method of assembling colloidal gold nanoparticles into macroscopic aggregates using DNA as linking elements [9]. The method involved semi-covalent bonds between non-complementary DNA oligonucleotides and the surfaces of two batches of gold particles
capped with R-SH (thiol) groups. Mucic et al. [25] had also described the construction of binary nanoparticle networks composed of colloidal gold. The same concept was extended to metallic nanowires and nanorods [26-28]. In one approach, metallic wires were formed by electroplating in porous alumina membranes with pores sizes of about 200 nm. Metallic rods, ranging from 1–6 μm in length were produced, depending on the electroplating conditions. In a series of publications, Braun and his colleagues [4-5] demonstrated the use of DNA as a template for the fabrication of nanowires. They reported DNA bridges between two thin-film gold electrodes using thiol attachment. Once a DNA bridge is formed between the 12–16 μm spacing of the electrodes, a chemical deposition process was used to deposit silver ions along the DNA. The result was a silver nanowire which was formed using the DNA as a template. Current–voltage characteristics were measured to demonstrate the possible use of these nanowires. Braun’s group had also extended its work in the use of DNA molecules as templates for self-assembled nanometer-scale conductive metallic wires using gold [5]. This was driven by the ability to manipulate and address, at nanometer-scale resolution, the underlying DNA sequences using well-known and standard molecular biology techniques. These techniques enable the precise localization of electronic materials (e.g. gold particles in this case) on DNA molecules thereby converting the insulating biological molecules into functionalized electronic components.

![Figure 2.2. (a) SEM of electrode gaps and the accompanying wiring. (b) SFM view of one electrode gap shows the two binding electrodes (top and center) [29].](image)

Csaki et al. [8] was one among the researcher who follow Braun's pathway. They fabricated metal structures as 12–16 μm spaced lines to attach 16 μm long Lambda-phage DNA (λ-DNA) for electrical characterization [19]. The structures, as shown in figure 2.2,
were defined on a silicon wafer by standard photolithography and deposition of a 100 nm Au layer by sputtering, followed by a lift-off process. The wafer was functionalized with 1 mM octadecyltrichlorosilane (ODTS) and cysteamine. The ODTS passivized the chips against non-selective adsorption of DNA while cysteamine would cause thiol modification of the Au electrodes. The thiol modification induced the positive charge on the Au structures which was used for electrostatic capture of the negatively charged DNA. Several DNA molecules were thus positioned in the electrode gaps. The number of the bound molecules was controlled by inserting the thiol modified chips into a flow chamber and subjecting to an oriented flow of λ-DNA solution. The chips were dried after DNA was captured and Scanning Force Microscope (SFM) imaging was done. The gaps were seen to be bridged by single thread-like structure, which putatively represented an individual DNA strand.

Figure 2.3. (A) Schematic and (B) AFM image of microfabricated Metal-Insulator-Metal structures [29].

Kleine et al. investigated the intrinsic DC conductivity of individual λ-DNA by ultra-sensitive low current–voltage-spectroscopy under ambient conditions and in controlled atmosphere on microfabricated metal–insulator–metal gap structures [29]. They produced Platinum electrodes on glass surfaces with gap sizes between 20 and 3000 nm by lift-off as shown in Figure 2.3. A droplet of λ-DNA was placed on the gap to be adsorbed over the structures. The meniscus forces during the drying process induced controlled orientation of the DNA, what they called as “molecular combing”. They measured $I-V$ characteristics in ambient conditions and in low humidity atmosphere.
Figure 2.4. (a) An image of the metal tip used by Fink and Sconenberger at Basel to probe a DNA bundle connected to a metal electrode on the left. (b) The I-V curve measured by the Basel team shows that DNA conducts well and has a resistance of about 1 MΩ [21]. (c) An arrangement of several single DNA molecules attached between two gold electrodes as studied by Storm's team at Delft. (d) Typical I-V curve demonstrated that DNA is an insulator [39].

However, the key question of whether DNA molecular wires are conductive by themselves or not continues to be contentious. As discussed widely by several authors, there are many recent reports covering the topic with a wide spectrum of results ranging from insulator-type (Fink and Sconenberger [21] is one of the examples), wide-band-gap semiconductor to super-conductive behavior of DNA molecular wires [31-43] as illustrated in Table 1. As we can see from the table (the number in the table refer to the reference number), most of earlier researches justify that DNA is a conductor only by consideration of π-stacking in the phosphate backbone structure. But then later experiments mostly said that DNA is an insulator before most of the later opinion proved that DNA behaves as a semiconductor.
Porath et al. [31] and Felica and Porath [32] were among the first who reported semiconducting behavior of DNA in February 2000, more and more result were in the support of the idea. They measured the conductivity of 30 base pairs (bp) 10.4 nm long poly(G)-poly(C) ds-DNA electrostatically trapped between two 8 nm spaced metal electrodes. Their results indicated that the strand behaved as a large band gap semiconductor with a voltage gap at low biases as shown in Figure 2.4. Ackley, Heller, and Edman had also proposed DNA-inspired self-assembly of active devices for assembling optical and optoelectronic components onto a host substrate using electrokinetics approaches [30].

Most of researchers have worked on DNA wires and DNA immobilization on various surface, none of these groups have investigated how to work on a platform that allows for testing and development of DNA as a biosensor or an electrical component [23].
Different approach has been established by Kassegne group with some improvements were addressed to remove the substrate effect and environment in a more direct and decoupled way by (i) introducing high aspect-ratio electrodes (>75µm height) from which double-stranded DNAs are suspended (away from the substrate) through thiol-gold covalent attachment of thiol-terminated oligonucleotides and gold electrodes and (ii) performing electrical characterizations in a dry-state [1]. This group was also tried to adopt the attachment of DNA to carbon nanotubes and apply the principle in a longer DNA wire. For this approach, pyrolized SU-8 has been used the generate carbon electrode [2]. The background to fabricate functionalize 3D carbon electrode via pyrolysis has been studied earlier by researchers in the field of Carbon MEMS (C-MEMS) [62] proven that the high temperature pyrolysis to SU-8 photoresist will generate carbon layer on the surface.

**Figure 2.5 The I-V curves measured at room temperature on a DNA molecule trapped between two metal nanoelectrodes [31]**
Further, in general, reports in the literature on electrical characterization in DNA-based bionanoelectronics are overwhelmingly limited to DC sources and short term I-V measurements in aqueous environment. This paper addresses this gap by reporting on both DC and AC impedance characterizations of DNA molecular wires in dry environment; thereby offering more insight to electrical characterization of DNA-mediated bionanoelectronics at three distinct levels, (i) modification of DNA attachment methods transform the approach from base pair self-assembly to phosphate backbone ligation resulting in much stronger attachment (ii) more accurate direct conductivity measurement of DNA molecular wires facilitated by suspension of the DNA away from the substrate, and (iii) AC impedance measurement of DNA molecular wires with the effect of temperature suggests an experimental evidence of temperature gating mechanism in charge transport through DNA wire we believe that this study will add a body of knowledge and experience in bio-inspired nano-manufacturing.
CHAPTER 3

DNA AS A MOLECULAR WIRE

Apart from its fundamental role in defining genetic code of living organism, DNA’s electronic properties have attracted interest and become the subject of much investigation over the past decade [45]. Moreover, recombinant DNA, the natural mechanism of gene mixing in cells, can be employed for the formation of molecularly accurate DNA junctions that can serve as templates for more complex electronic devices [46]. DNA could also be useful in nanotechnology for the design of electric circuits, which could help to overcome the scaling down limitations that classical silicon-based electronics is facing in the coming years. In this case, understanding charge transport mechanism on DNA is the most important subjects in defining the electronics properties of DNA. While recent research in electron-transport mechanism on a double strands DNA seems to converge into a consensus, experiments in direct electrical measurements on a long DNA molecular wire still lead to a conflicting result [47].

3.1 DNA FUNDAMENTALS

DNA is the basic building block of life. Hereditary information is encoded in the chemical language of DNA and reproduced in all cells of living organisms. Since the discovery of the peculiar structure and characteristic, it has mesmerized many scientists in diverse field of biology, chemistry, physics, Biomedical engineering and most recently nanotechnology. In the following chapter, we will cover some of its fundamental properties.

3.1.1 DNA Structure and Properties

The double-stranded helical structure of DNA as described by Watson and Crick [48] is the key to its use in self-assembly applications. Each strand of the DNA is about 2 nm wide and composed of a linear chain of four possible bases (adenine, cytosine, guanine, and thymine) on a backbone of alternating sugar molecules and phosphate ions. Each unit of a phosphate, a sugar molecule, and base is called a nucleotide and is about 0.34 nm long. The
specific binding through hydrogen bonds between adenine (A) and thymine (T), and cytosine (C) and guanine (G) can result in the joining of two complementary single-stranded DNA to form a double-stranded DNA. There are two hydrogen bonds between A-T pairs and three hydrogen bonds between G-C pairs. The phosphate ion carries a negative charge in the DNA molecule, which results in electrostatic repulsion of the two strands. In order to keep the two strands together, double strands DNA must be stored in buffer solution where positive ions must be present in the solution to keep the negative charges neutralized.

Figure 3.1. Primary Structure of Watson and Crick DNA double helix DNA [48].
DNA’s chemical structure results in π-bonds (attractive and non-covalent interactions between aromatic rings) along the back bone in the phosphate and sugar molecules and in several locations in each of the bases. It is believed that electrons travel along the π-bonds to conduct electricity [50]. DNA is prevalent in nature, present in all organisms and can be extracted from cell and sequenced or synthetically generated for specific base pair sequences. It is small, having a diameter of only 2 nm [50]. It can be made or found at any length. Furthermore, under appropriate condition, DNA also has the ability to self-assemble into nanostructures. These properties of DNA, namely conductive, nano-scalable, and self-assembly, make DNA a promising component in bio-nanoelectronics.

**Figure 3.2. The Watson and Crick base pairs, A:T and G:C [48].**

**Figure 3.3. Illustration of π-bonds, attractive and non-covalent interactions between aromatic rings. In DNA, it occurs between sugar rings along the phosphate backbone [20].**
3.1.2 Melting Temperature

A discussion on the effect of melting temperature ($T_M$) of the DNA will be very useful aspect for this research, especially to analyze the effect of temperature to DNA wire. The joining of two complementary single strands of DNA through hydrogen bonding to form a double-stranded DNA is called hybridization. If a double-stranded DNA is heated above a certain temperature, the two strands will start to separate (denature) and eventually at some point will be fully separate into two single strands (complete denaturation) [49].

![Figure 3.4. DNA denaturation and annealing as a reversible process [49].](image)

The center temperature of this transition is called the melting temperature, $T_M$, which is a sensitive function of environmental conditions such as ionic strength, pH, and solvent conditions. As the temperature is reduced, the two strands will eventually come together by diffusion and re-hybridize or re-anneal to form the double-stranded structure. These properties of the DNA can be utilized in the ordering and assembly of artificial structures if these structures can be attached to DNA [49].
3.2 DNA ELECTRONICS

Electron excitations and motion of electric charges are well known to play a significant role in a wide range of macromolecules of biological interest [3]. Electron transfer involving the DNA double helix is thought to be important in radiation damage and repair and also in biosynthesis; the double helix may mediate charge transfer between different metal complexes [4-7].

3.2.1 Charge Transport Mechanism in DNA

The possibility of long-range electron transfer through a DNA molecule has intrigued many researchers for a long time. Radiation biologists had invoked this concept almost 40 years ago to account for what was considered unusually high conductivity of DNA. However, later studies showed this to be a consequence of water molecules present due to water in the medium or of the charge mobility due to ions outside the duplex [51]. While studies based on electron spin resonance and luminescence methods provided support for long-range electron tunneling those based on the pulse radiolysis technique indicated that electron tunneling is restricted to around five base pairs [52].

Current theoretical models assume DNA molecule to behave as a one-dimensional aromatic crystal with π-electron conductivity. This suggest that the DNA structure is ideal for electron/hole transfer, and charge transference through DNA proceed along a one-
dimensional pathway constituted by the overlap between $\pi$-orbitals in neighboring base pair [20]. Eley and Spivey [54] reported conductivities in order of $10^{12}$ ($\Omega$ cm)$^{-1}$ and energy gaps ($\Delta E$) of about $2.42 \pm 0.05$ eV at 400K. While emphasizing lack of knowledge on RNA structure at that time, they reported similar experimental value for RNA. Snart, in 1973, reported a similar and reproducible value of energy gap (2.4 eV) that was affected by UV radiation [53]. Snart’s measurement method was very similar to the one employed by Eley and Spivey. These can be considered as the starting points in the quest for DNA conductivity.

Murphy from Barton Research Group of Caltech carried out seminal studies to elucidate electron transfer chemistry mediated by DNA in 1993 [54]. Their work have sparked a lot of debate about the mechanisms of charge transport through the double helix. Barton and co-workers performed experiments that suggest long range electron transport in DNA [56]. The donor and acceptor molecules were intercalated on the DNA strands. When donor was photo-excited, the fluorescence of the donor quenched due to electron transfer to acceptor. The donor and acceptors were basically metal complexes. Their results showed very rapid transfer of carrier over $>40$ Å via $\pi$-stacked base pairs. However other researchers disputed their results, as reproduction of the result with other donor and acceptor candidates proved problematic. The starting point of the controversy came very next year when similar experiments, Brun and Harriman used organic donors and acceptors [57] and concluded that charge-transfer rates drop off fast with the increase in the length of the DNA; result contradicting the work of the Barton group.

On theoretical side, Jortner et al. proposed two models of DNA transport; direct-step holes transport from one base pair to another, and, a multi-step charge transport through the base pairs [58]. They proposed that one of these mechanisms was predominant in charge transfer depending on the base sequence. The first mechanism is the direct tunneling of electron through the phosphate backbone. This mechanism is highly dependent on the overlapping $\pi$-orbital which the electron is tunneling through. The second mechanism is a multi-step charge transport through the base pairs which exhibit weak dependence on the separation distance between the donor and acceptor. Ly et al. studied mechanism and distance dependence on radical anion and cation migration [59]. They attached an acceptor, anthraquinone (AQ), to the central position of a DNA duplex with a GG sequences at equidistant positions on both side of AQ. They characterized the trapping of radical cations at
AQ site by photochemically activate it. They considered two extremes of charge transfer; DNA as molecular wire and the hole hopping mechanism, both concepts previously proposed by Jortner et.al. They suggested that the structural flexibility of DNA dictates a mix behavior of hole-hopping and continuous orbital mechanism. Such disturbance in DNA most likely results in the reduction of intra-base distance and unwinding of DNA giving way to increase \( \pi \)-electron overlap and shift of internal charges inside hydrogen bonds. All these effects constitute the polaron-like distortions and in their model polaron migrate by hopping. Again such hopping will depends on intercalated based sequence. Giese and Wesley in 2000 verified the above two mechanisms by experiments [60]. They used GGG as an electron donor, i.e. acceptor for hole. They injected a hole at a modified G base with \( n \) number of AT base pair away from this triple G. Introduction of a mismatch at GC bp resulted in a strong decrease in the charge transport in ds-DNA indicating the positive charge migrated by reversible tunneling reaction steps between neighboring G bases. There was a coherent super-exchange reaction (single step tunneling) and a thermally induced hopping process for long-range charge transfer, slightly influenced by the number of intercalated AT. Generally, in hopping mechanism, the G base is considered the most favorable for landing of holes or trapping, basically because it has the least ionization potential among four bases (\( G < A < C < T \)), independent of neighboring effects.

\[ \text{Figure 3.6. Simplification of DNA charge transport mechanisms: Super-exchange or tunneling (left) and charge hopping (right) [60].} \]

Yu and Song modeled DNA as 1-D disordered system with electrons’ transport occurring between localized states as variable range hopping [61]. The disorderliness of a DNA system stems from a random base pair sequence, resulting in localized electronic states.
These localized states in the sequence will present the candidate landing site for the hopping electron, while such localization would be enhanced by structural changes in DNA with temperature. This result can be explained by invoking a “variable-range hopping” model with a temperature dependent localization length. Here, the probability of hopping is dependent on two mechanisms, both tunneling (characterized by the localization length) and a thermally activated hopping. Thus, the most probable distance for hopping is due to the competition between these two mechanisms. If this is the case, there is a critical temperature above which the most likely hopping distance becomes smaller than the distance between bases. Above this temperature, the hopping mechanism can only be thermally activated. This fact alone is enough to describe a transition from weak temperature dependence to strong temperature dependence. They did fit the theory with experimental conductivity data from λ-DNA. This variable range hopping mechanism is somehow consistent with the two possible mechanisms Jortner et al. and Ly et al. proposed.

Table 2. Summary of Researches in DNA Charge Transport Mechanism
A number of theoretical models have been proposed to explain the conductivity and charge transfer through DNA. There is higher probability for presence of more than one mechanism in any given experiment. Table 2 gives the idea of previous publication related to the charge transport mechanism and Table 3 classify them based on their detection methods for the experiments. The ideal model should take care of the effect of DNA structure, thermal motion of charges as described by classical models, effects of cations in the solution, on the structure and on the Coulomb charging, temperature, inter-molecular and intra-molecular attraction and repulsion, effect of contacting conductors and the chemistries used to contact the DNA, etc.

### Table 3. Summary of Studies in DNA Charge Transport Mechanism

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Detection Experiment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron Tunneling</td>
<td>Electrostatic trapping</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td>Microwave conductivity</td>
<td>[6]</td>
</tr>
<tr>
<td></td>
<td>Kinetic rate equations</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td>Scattering matrix formalism and Büttiker’s dephasing model</td>
<td>[31], [45], [59]</td>
</tr>
<tr>
<td></td>
<td>ultrasensitive low current–voltage-spectroscopy</td>
<td>[29], [37]</td>
</tr>
<tr>
<td></td>
<td>Resonance Cavity</td>
<td>[106], [52], [110], [113]</td>
</tr>
<tr>
<td></td>
<td>Luminescence</td>
<td>[52], [55], [56]</td>
</tr>
<tr>
<td>Charge Hopping</td>
<td>Yield measurements of oxidative damage using gel electrophoresis</td>
<td>[52]</td>
</tr>
<tr>
<td>Not Specify</td>
<td>Physical measurements of current flow</td>
<td>[20], [37]</td>
</tr>
<tr>
<td></td>
<td>X-ray diffraction and reflection high-energy electron diffraction (RHEED)</td>
<td>[42]</td>
</tr>
</tbody>
</table>

### 3.2.1 DNA Molecular Wire in Nanoelectronics

There has been a tremendous interest in recent years to develop concepts and approaches for self-assembled systems. While significant work continues along this
direction, it has also been recognized that the exquisite molecular recognition of various natural biological materials can be used to form a complex network of potentially useful particles for a variety of optical, electronic, and sensing applications [63-64]. This approach can be considered a bottom-up approach rather than the top-down approach of conventional scaling. DNA is a particularly promising candidate to serve as a construction material in nanotechnology. Despite its simplicity, the highly specific Watson-Crick hydrogen bonding allows convenient programming of artificial DNA receptor component. The power of DNA as a molecular tool is enhanced by automated methods and by the PCR technique to amplify any DNA sequence from microscopic to macroscopic quantities. Another attractive feature of DNA is the great mechanical rigidity of short double helices, so that they behave effectively like a rigid rod spacer between two tethered functional molecular components on both ends. Moreover, DNA displays a relatively high physicochemical stability. Finally, nature provides a complete toolbox of highly specific enzymes that enable the processing of the DNA material with atomic precision and accuracy.

The concept of DNA-mediated self-assembly of nanostructures has also been extended to metallic nanowires [4, 27-28]. Braun et al. have utilized DNA as a template to grow conducting silver nanowires [4, 29] as we can see in Figure 3.8 above. The basic assembly scheme for constructing a silver nanowire attached to two gold electrodes is outlined in Fig 3.7 where two gold electrodes separated by a defined distance (12-16μm) were deposited onto a glass slide using photolithography. The gold electrodes subsequently were modified with non-complementary hexane disulfide modified oligonucleotides through well-established thiol adsorption chemistry on Au. Subsequently, a fluorescently labeled strand of DNA containing sticky ends that are complementary to the oligonucleotides attached to the electrodes is introduced. Hybridization of the fluorescently tagged DNA molecule to the surface-confined alkylthiololigonucleotides was confirmed by fluorescence microscopy. This work is a proof-of-concept demonstration of how DNA can be used in a new type of chemical lithography to guide the formation of nanoscale circuit.
Due to its unique recognition capabilities, physicochemical stability, mechanical rigidity, DNA is a promising material for “biomolecular nanotechnology”. Though significant progress has been made, the study of DNA-based nanostructures is still at its early stage. The catalytic, electrical, magnetic, and electrochemical properties of such structures have not yet been systematically investigated, and they, therefore, represent new frontiers in this field. It is anticipated that new phenomena and useful structures will continue to emerge over the next few years. Advanced study in this field will not only provide valuable fundamental information about the collective physical and chemical properties of nanoparticles and DNA, but also may provide access to new and useful electronic and photonic materials applicable to the industry.
CHAPTER 4

BIONANOELECTRONIC PLATFORM FOR DNA ATTACHMENT

Generating the proper bionanoelectronics platform involves several steps starting with fabrication of microelectrodes using photolithography methods followed by DNA attachment to the microelectrodes followed by optical and electrical characterization. The 3D microelectrode set which is typically micromachined to a height of 10-100µm has four bump-pads for resistance measurement. A similar microelectrode design (albeit with some key differences in material and aspect-ratio, as well as substrate material) was reported by Braun et al. [5].

The conceptual illustration of this bionanoelectronics platform with DNA molecular wire attached to high aspect-ratio metal electrode through oligo-mediated thiol-gold covalent attachment protocol is shown as in figure 4.1. λ-DNA was used primarily due to its typical dimensions of 2nm diameter and 10-15µm length which corresponds to the gap between the microelectrode sets.

Figure 4.1. Schematics of 3-D bio-nanoelectronics platform where oligos A & B are attached to the microelectrodes followed by their hybridization with thiolated λ-DNA. Note the four pads used in the chip; these will be used for four-point probe test.
This type of DNA is derived from a bacteriophage called lambda which typically inhabits E. coli bacteria and is easy to isolate in large quantities and cut to fragments. In addition, there is a body of knowledge in the use of λ-DNA in gel electrophoresis as well as bionanoelectronics [4,21,32-34,37,41]. In the following sections, we will describe the lithography process and discuss in detail the DNA attachment method.

**4.1 DESIGN AND FABRICATION OF MITHRAS ELECTRODE**

The 3-D chip architecture is made of a gold electrode layer on top of a negative photoresist structure of at least 10µm height on SiO2 substrate. The high aspect-ratio 3-D electrodes perform the primary function of suspending the DNA high above the substrate eliminating non-specific binding and substrate effect on resistance measurements (which has been a major unknown and constraint in almost all previous reports).

Figure 4.2. (a) Image of an array of 3D bio-nanoelectronics platform consisting of microelectrodes, traces, and bumps pads. (b) A typical chip after microfabrication. In a typical design shown in (b), the spacing in between the microelectrode set varies from 8µm-15µm. The height of the chips is between 50-100µm. There are four bump pads for resistance measurement. Gold is sputtered only on the top surface.

The following sections describe, in detail, how the Mithras design as shown in figure 4.3 was manufactured using SU-8 photoresist and standard photolithography procedures [66] using the mask as shown in figure 4.4 and followed by gold metal lift-off.
Figure 4.3. Detail image of Mithras microelectrode

Figure 4.4. Mithras electrode mask for negative lithography
4.1.1 Microfabrication Process

To start the lithography process, the silicon wafer was washed with water, acetone, isopropyl alcohol, and water and then dried with an air gun. To ensure silicon wafer was completely dry, the wafer then placed on a hot plate for two minutes at 120°C for dehydration bake, and then taken off the hot plate and allowed to cool for 15 minutes. Then the silicon wafer was placed on the chuck of the spin coater and a layer of SU-8 was deposited. The SU-8 was spin coated at 3000 rpm for 45 sec, then removed from the chuck and place on the hotplate at 45°C. The temperature was increased up to 95 degrees Celsius over a period of 40 minutes, held at 95°C for 5 minutes and then allowed to cool for 30 minutes.

Table 4. Microchem Process Guidance for SU-8 10 Photoresist

<table>
<thead>
<tr>
<th>SU-8</th>
<th>Thickness (in μm)</th>
<th>Spin Speed (in rpm)</th>
<th>Minutes to Pre-Bake @65°C</th>
<th>Minutes to Soft bake @95°C</th>
<th>Minutes to PEB @65°C</th>
<th>Minutes to PEB @95°C</th>
<th>Development Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>SU-8 10</td>
<td>5**</td>
<td>4400**</td>
<td>10**</td>
<td>10**</td>
<td>5**</td>
<td>5**</td>
<td>1.2**</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3000</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>2000</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1000</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

Figure 4.5. Microchem suggested process and UV dose for SU-8 10 photoresist [65].
During the cooling process, the UV lamp was set up to expose 8.08mW/cm². Once the wafer was cooled, the wafer was set up under the UV lamp with the mask placed over it. The wafer was then exposed for 30 seconds and the mask was removed and the wafer was placed back on the hot plate for post-baking.

Post baking was done at 50°C to 100°C over a period of 30 min and then allowed to cool for 15 minutes. Figure 4.3 summarize the lithography process described above. Platinum was then sputtered on top of the SU-8 layer as an intermediate layer followed by gold sputtering, both metal have thickness of 6 nm. As the photoresist has not been develop, we need to protect the wafer from light exposure that can attack the photoresist prior to metal sputtering. In this case the metal sputtering will be done in the dark.

Figure 4.6. Photolithography process map [44].

Finally, in order to remove the portions of the polymer that were not exposed to UV light and thus not cross-linked together with metal layer on top of it (metal lift-off), the wafer was placed in SU-8 developing solution. Developing time was different for each chip depending on the exact thickness of the SU-8 layer and the size of the wafer itself. However,
for features that were expected to be about 10 microns in height, development time was not expected to take more than 5 minutes. Therefore, after about 1 minute in developer, the wafer was rinsed with isopropanol. If a white film showed up, then the wafer would need to be rinsed with acetone and placed back in developing solution for no more than 15 seconds. If not the wafer was rinsed with water and dried with the air gun and taken to the microscope for imaging to ensure that there was no need for further development.

**4.1.2 Mithras Chips Imaging**

For visual inspection and for ensuring proper development of the microelectrodes, imaging was done using 2-D and 3-D lenses on a Hirox optical microscope.

Figure 4.7. Chip #9: negatively fabricated with layers of SU8-10 + Au on Pt, Gap = 11.60 μm, thickness = 10 μm.
Figure 4.8. Actual image of Mithras chips made with lithography using SU8-10, 6nm gold sputter on a silicon wafer with oxide layer

We also utilized Scanning Electron Microscope (SEM) to get a confirmation of conductive layer on the surface of electrode and more detail measurement of the gap.

Figure 4.9. SEM Images of Chip #9: negatively fabricated with layers of SU8-10 + Au on Pt, gap = 11.60 μm, thickness = 10 μm.
4.2 DNA ATTACHMENT METHOD

In its simple form, the attachment process is based on first attaching the annealed two oligonucleotides of complimentary sequence but one of them has the two thiol-modified ends to the gold microelectrodes followed by attachment of blunt λ-DNA to these strands of oligonucleotides via phosphate backbone ligation. This function is based on the self-assembly property of DNA. In the following sections, this process is described in detail.

4.2.1 Oligonucleotides Preparation and Annealing

Two oligonucleotides (Oligonucleotide A: 5'-GGG CGG CGA CCT /3ThioMC3-D/ -3' and Oligonucleotide B: 5'-AGG TCG CCG CCC -3') from Integrated DNA Technologies, Coralville, Iowa) were individually diluted with Tris-EDTA to generate two 1 μM solution of oligonucleotides. It is done by twice of 10x dilution and one time of 5x dilution in a room temperature. Equal volume of both oligonucleotides (normally we use 20 μL each) was then mixed in a microtube and placed in a water bath that is heated to around 105°C for 5 min (assume that the temperature inside the tube will be around 95°C). The tube then being removed from the water bath and allow it to cool to room temperature before we can store it in a refrigerator at 4°C until use.

4.2.2 Oligonucleotide Reduction

To use the free thiol(–SH), the disulfide linkage must be reduced with TCEP gel. It is suggested by the manufacturer [67] to mix composition the TCEP with the annealed oligonucleotides solution in of 1:1 composition by volume for the reduction. The process started with taking 40 μL of TCEP solution, vortex it and leave for 5 minutes for the slurry to condense and separate from the supernatant. The supernatant then been removed from the condensed slurry. To have similar volume, 40 μL of annealed oligonucleotides solution was then taken and mixed with the TCEP slurry. It was then incubated at room temperature for 10 minutes to have a complete reaction and the solution to separate from the slurry. Then the supernatant was taken as the reduced oligonucleotides and should be used immediately as the free thiol will remain active only in about 10 minutes duration.
32

Figure 4.10. Reduction of a typical disulfide bond by DTT via two sequential thiol-disulfide exchange reactions.

4.2.3 λ-DNA End Repair

The λ-DNA (New England Biolabs, Ipswich, MA) was suspended in TE solution (10mM Tris-HCl, 1mM EDTA) was obtained. The λ-DNA contains 48,502 base pairs with its 5´ strand overhanging its 3´ strand by 12 bases. These single stranded overhangs have to be repaired to get a blunt end DNA using DNA end-repair kit from Epicentre [68]. Standard 30μl reactions were used to get end-repair up to 12.5 μg of DNA (DNA original concentration is 0.5 μg/μl). The reaction can be scaled up or down as necessary. It started with mixing 25μL of linear, sheared and concentrated λ-DNA with 3μl of T4 Ligase buffer, 1 μl of 10mM dNTPs, 0.5 μl of T4 Polymerase and 0.5 μL of T4 PNK in a 2 ml microfuge tube. Then after incubated them at room temperature for 45 minutes, reaction should be stopped by heating at 70°C for 10 minutes. The end-repaired DNA can be used for DNA ligation without purification. The blunt-end ligation reaction can be performed for 45 minutes to 2 hours at room temperature. For the usage, some series of serial dilution was done to have DNA in many different concentrations (500, 50, 25, 5, 2.5, 1, 0.5 and 0.25 ug/μl) and they can be stored at 4°C until it use.

4.2.4 Oligonucleotide and DNA Attachment via Ligation

Before attachment, plasma etching is necessary to be done to remove the effect of surface to small volume drop. As illustrated in figure 4.10 we can see that the surface effect
avoided the solution to spread on the silicon wafer substrate. Plasma etch was done at 75 Watt for about 7 second.

![a](image1.png) ![b](image2.png)

**Figure 4.11.** A drop of solution on top of mithras electrode. Without plasma etching, the drop creates bubble and not spreading (a), while after plasma etched the solution spread freely on the surface (b).

Using a small volume micropipette, a 1 μL droplet of reduced oligonucleotides was deposited on the center of the 3D microelectrode structure while viewing the electrodes through an optical microscope. The chip was soaked for 10 minutes and then rinsed with DI water. After oligonucleotides attachment, 1 μL solution of λ-DNA was dispensed on the chip covering the region where the oligonucleotides are located. A ligation mixture was then prepared based on the manufacture guide. Standard DNA ligation mixtures consist of 1 μL of DNA ligase, 4 μL of DNA ligase buffer and 15 μL of DI water as instructed by manufacturer [69]. The formula can also be scaled up or down based on necessity. To activate the ligation, 1 μL of the ligation mixture was then dropped on the center of microelectrode and then set aside at room temperature for 60 minutes. Electrical bias should be performed every 15 minutes to attract DNA closer to the electrodes. Finally, the chip was then washed and rinsed with DI water and set aside to dry at room temperature.

**4.2.5 Attachment Imaging & Optical Characterizations**

Fluorescence occurs when there is less than a microsecond time delay between photon absorption and emission. Fluorescent microscopy uses this property by exposing a sample to light at its excitation wavelength and using a sequence of filters to detect the emission spectra at appropriate wavelength [70]. While some materials naturally fluoresce
under certain wavelength, most biomolecules require a fluorescent stain in order to induce this property. DNA stains can bind to DNA along the backbone (external binder) or between the bases (intercalation), or across the bases (groove binders) depending on which stain is being used.

Once the DNA stain is bound to the DNA, a fluorescent microscope can be used to excite and view the emissions from the stain. The excitation and emission wavelengths depend on the type of stain used. The fluorescent stain used in this study was SYBR Safe Stain from Invitrogen. This stain has excitation wavelengths between 400 and 530 nm and emission wavelengths between 500 and 700 nm. However, optimal imaging conditions exist where excitation and emission wavelengths overlap. For SYBR safe stain, this is at 470-530 nm [70].

![SYBR® Gold binds to dsDNA and illuminate under fluorescent microscope](image)

**Figure 4.12. SYBR® Gold binds to dsDNA and illuminate under fluorescent microscope [70]**

For each electrode, 0.5 uL of DNA Safe Stain was diluted in 2mL of DI water. The electrode was placed in a beaker with the diluted stain and agitated for 10-30 minutes using a magnetic stir bar and stir plate. The electrode was then removed from the solution, rinsed with DI water, and placed on a microscope slide for imaging. The microscope (figure 32) was then set up to use a blue light for excitation and a green filter.

### 4.3 ATTACHMENT OPTIMIZATION WITH MICROFLUIDICS

Microfluidics is defined as the science and technology of manipulating small volumes of liquid (<10⁻⁶ liters) using sub-millimeter channels (10⁻⁶ - 10⁻³ meters) [71]. Controlling liquids on this scale offers a number of important advantages and applications. For example, microfluidics has a wide range of applications in analysis, particularly bio-analysis [72].
Biological samples are usually difficult to obtain, challenging to store, potentially hazardous during disposal, typically available only in low concentrations, and complex in nature. Microfluidics involves small channel size, which reduces sample requirements, decreases reagent consumption and minimizes waste. Microfluidics devices are reported to have higher throughput (through parallel operations), lower levels of contamination (using disposable units) and offer better sensitivity than is the case for traditional bench-top instruments. Microfluidics also offers the promise of near-patient (or point-of-care) diagnostics which is essential for use in environments with limited resources and infrastructure, such as battlefields and remote areas. In addition, microfluidics technologies have proven to be useful new tools for biological studies and engineering [72-75].

Unlike other microfluidics systems that are created for miniaturization and lowering the operation cost for research, utilization of microfluidics in this study has some very unique reasons. The main reason is to be able to deliver the DNA solution as close as possible to the targeted location for attachment, which is in the 10 micron gap in between two electrodes. The second most important reason is that by creating a circulation of the DNA solution, we can increase the chance of getting DNA bridges connecting both ends of the electrodes. The following section will discuss in detail the design, fabrication and experiment setup using microfluidics.

4.3.1 Soft Lithography for Microfluidics Chips Fabrication

Soft lithography consists of a set of polymer-based microfabrication techniques. These techniques were developed by George Whitesides and his coworkers in order to complement photolithography [76-77]. The core concept of soft lithography is the replication of microstructures made by photolithography or other techniques onto a soft polymer stamp, followed by the use of the stamp as a patterning tool or for the purpose of making a channel system. A common method of making an elastic stamp is illustrated in Figure 4.12 (a). Pre-polymer solution is cast on a patterned (silicon) master, cured and peeled off. The resulting stamp bears a surface relief structure that is the negative of what is on the master. The microcontact printing technique (Figure 4.12 (b)) uses the relief pattern on the stamp to form ink patterns on the surface of substrates by means of contact. Self-assembled monolayers (SAMs) constitute an excellent ink for this purpose due to its crystalline-like structure and
nanometer thickness. The SAMs patterns formed by thiol/thiolated or silane on gold or silicon surface can serve as an etching mask for further patterning. In addition to patterning, the elastic stamp can also seal with other substrates to form microfluidics channel systems (Figure 4.12 (c)). Once a microstructure master has been made using photolithography or other techniques, soft lithography can be used to create numerous polymer replicas quickly and easily in ambient laboratory environments without the use of specialized equipment, which produces a significant decrease in fabrication cost and lowers barriers to enter.

Polydimethylsiloxane (PDMS) is the polymer most commonly used to make microfluidics devices. Several properties of PDMS make it a particularly suitable material for biological applications. PDMS is transparent down to ~ 300 nm and low in background fluorescence, which makes PDMS-based microfluidics devices compatible with optical and fluorescence microscopy. PDMS is inert in water and is biocompatible, which makes it useful for cell studies. Another useful property is that biologically important gases (e.g., O₂, CO₂) can pass PDMS easily, which makes on-chip cell growth possible [78].

PDMS can form reversible or irreversible bonding with many substrates that are used to make microfluidics devices. Glass, polystyrene, and silicon are widely used in biology, and PDMS can reversibly bond with these materials. The reversible bonding between PDMS and these materials is driven by van der Waals’s force, which is strong enough to withstand 5 psi pressure. PDMS can also be used to form irreversible bonding with numerous materials (e.g., clean glass, silicon, and polystyrene) when its surface is activated by oxygen plasma, UV/Ozone, or Corona [78]. These treatments generate -OH groups on the PDMS surface, which can form covalent bonding with other materials. In some cases, PDMS needs to bond with porous membranes (e.g., polycarbonate membrane) in order to make multilayer channels. These hybrid channels are useful for making immunoassays, making gateable nano- or micro-fluidic interconnections [79-80], for studying the flux of platelet agonists into flowing blood [81] and pathogen detection system [82].

Special surface treatments are required for a permanent bonding between PDMS and polymer membranes. Methods such as applying a thin layer of epoxy or PDMS pre-polymer glue should be conducted with great care in order to avoid microfluidics channels becoming blocked. A recent study used a home-built initiated chemical vapor deposition instrument to coat PDMS and its bonding substrate with a layer of epoxy nano-adhesive [83].
study involved coating polymer membrane with 3-aminopropyltriethoxysilane and claimed that an irreversible bond with PDMA was formed [84].

Figure 4.13. A schematic illustrating the fabrication of a PDMS stamp (a), which can be used to pattern molecular ‘ink’ through Microcontact Printing (b) or sealed with a given substrate to form a microfluidics channel system (c). [71]

4.3.2 Design and Microfabrication of Bali Mithras

Regular microfluidics channel normally have two components which are the flexible stamp made of PDMS and a substrate that will act as a seal, and the most common substrate is a glass slide. It means that only one time lithography required in producing the unit. In this experiment, two sets of lithography were required as the first lithography will be for the fabrication of the substrate that will have the Mithras electrode with gold layer on top of the surface. Second lithography using the mask as shown in figure 4.14 was to create the master for PDMS channel fabrication. The integration between those two layers will be similar with the regular microfluidics fabrication utilizing oxygen plasma bonding.
Figure 4.14. Three-dimensional illustration of Bali Microfluidics design. It consists of the Bali Mithras electrode (a) and PDMS channel (b). Figure (c) show the side view and the final integration is illustrated by figure (d).

Figure 4.15. Mask design for Bali Microfluidics channel
The microfabrication process of Bali Mithras chip is almost similar with the regular Mithras chip using negative lithography and SU8-10 as the photoresist. The major difference in Bali Mithras is that we need the bump pads to be stretch out wide so it is not covered by PDMS channel. It will give us flexibility to access the bump pads for electrical characterization during the experiment.

![Figure 4.16. Design of Bali Mithras Electrode](image)

Figure 4.16 show the actual Bali Mithras before and after bonding and the view inside the channel where the electrodes located is shown in figure 4.17.

![Figure 4.17. Bali Mithras microfluidics chip, before and after bonding](image)

While the second lithography for creating the master channel was using SU8-100. The main reason is because the channel width was 100 micron and having ratio 1:1 will be very good for the channel to provide space for the fluid to flow but not too wide to cause the channel to collapse. The process was quite similar with the negative lithography for the Mithras chip. It started with cleaning process using water, acetone, isopropyl alcohol, and water and then dried with an air Followed by dehydrating baking at 120°C for 2 minutes then taken off the hot plate and allowed to cool for 15 minutes. The spin coating to deposit layer
of SU-8 was at the same rate, 3000 rpm for 45 sec, then removed from the chuck and place on the hotplate at 45°C. Soft baking also use similar process, which was heated up to 95°C over a period of 40 minutes, held at 95°C for 5 minutes and then allowed to cool for 30 minutes. UV exposure also set at 8.08mW/cm² for 30 seconds. Post bake also ramping up from 50°C to 100°C for 30 minutes. In this case, we do not need a sputtering but directly develop the feature in ultrasonic shaker for about five minutes.

![Image](image_url)

**Figure 4.18. View of the electrodes inside the microfluidics channel**

To create the bonding between the electrode and the channel, plasma etching was utilized with energy of 75 Watt for about 7 seconds. After the plasma energy creates some free oxygen on both surface of the electrode and PDMS, it can be attached to each other based on the oxygen bond between both surfaces.

### 4.3.3 Microfluidics-assisted DNA Attachment

The utilization of microfluidics in the DNA related experiments has always been related to the DNA microarray where the microfluidics were playing major role in the mixing and hybridization of DNA [85]. In this experiment, we utilize the microfluidics only as a tool to deliver and circulate the DNA solution using peristaltic pump as the source of force. There is a slight modification to the DNA attachment protocol since for this microfluidics experiments, the main difference is that the thiol bond reduce will be done the last after ligation inside the tube instead of on the chip. The process started similar as the open reservoir attachment with the dilution and annealing of oligonucleotide A and
oligonucleotide B. At the same time, λ-DNA also been prepared for end repair using the similar end repair procedure. DNA ligation kit was also being prepared following the same preparation protocol. In the end 1 µl each of those three components (annealed oligonucleotides, blunt end DNA solution and the ligation mixture) were mixed together followed by dilution of the final mixture to have DNA solution at some different concentrations. Reducing the solution to activate the free thiol end of the oligonucleotide should be done right before injecting the solution to the microfluidics system.

This microfluidics experiment was using peristaltic pump as the flow source. The principle of peristaltic pump is similar with the utilization of Coriolis force produced by centrifugal pump to provide movement inside the channel [87]. As shown in figure 4.19, two sets of 1 mm tubes were used to connect the channel with the main tube from the peristaltic pump. The flow rate was set base on the manufacturing guide [86] to provide the desirable flow rate. During the experiment, DNA solution delivered at the lowest flow rate possible which was 0.01 rpm or 0.42 µl/min with direction changed every minute. During the flow, a DC measurement was also done simultaneously to give an indication of the conductivity inside the tube. The ideal condition will show “overflow” when there’s nothing conductive connecting the gap, then resistant should be measured in kΩ range when we get the DNA
solution in the gap and MΩ when we get bridge in the end. Experiment will be stop when we get the MΩ reading and followed by drying process before electrical characterization.

**Table 5. Peristaltic Pump Flow Rate Based on Manufacture Guidelines [86]**

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<tr>
<th>Mat'l</th>
<th>Tubing Length, (In)</th>
<th>Cat. No.</th>
<th>ID, mm</th>
<th>Max flow at 48rpm (mL/min)</th>
<th>Color Code</th>
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<td>PVC</td>
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<td>39-620</td>
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<td>0.33</td>
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CHAPTER 5

EXPERIMENTAL RESULT

Getting DNA attached to the gold electrodes was not the final goal for this study; we utilize the DNA attachment as a tool for further study especially in understanding the mechanical and electrical properties of the DNA wire. Based on that consideration, the experiment result will be presented in three parts. The first part will discuss in detail of the result of DNA attachment to the gold electrode, including visual image obtained from fluorescent microscopy, AC electrical characterization, and the Electron Dispersion X-ray Spectroscopy (EDX) for material characterization. Optimization of DNA concentration will be presented in the second part. Finally, the third part will show in detail the result of the temperature effect to the DNA molecular wire that will include the hysteresis and renaturation process.

5.1 DNA ATTACHMENT TO GOLD ELECTRODE

Fluorescent microscope was utilized as the main tool to capture images of attached λ-DNA between two gold electrodes using oligo-mediated attachment as well as some control experiment. This method is based on the binding of fluorescent tag in between the base-pairs of dsDNA.

5.1.1 Visual Result with Fluorescence Microscopy

Some positive control experiments were used to verify each major step in DNA attachment method and also to confirm the existence of λ-DNA between the two electrodes. The first control was to verify if the annealing of the oligos was successful.
Figure 5.1. Oligonucleotide annealing control experiment. Figure (a) show a drop of oligo A and oligo B without annealing and figure (b) was captured after annealing.

The indication of this success is that the annealed oligos should illuminate under fluorescent microscope while the single strand oligonucleotides were not (as shown in figure 5.1). The second control was for the reduction of the oligonucleotides. The success of the process will result in a free thiol end on the oligonucleotide that will be able the create a thiol bond with the gold electrode. The control experiment was to visualize the chip after a drop of reduced oligonucleotide. As we can see in figure 5.2, the oligos were attached to the gold electrode (indicate by the illumination on the edge of the electrode) but not connecting the electrode as the oligonucleotides were just some very short strands of DNA.

Figure 5.2. Oligonucleotide reduction control experiment.
Then finally after the experiment, oligos and λ-DNA attachment on gold electrodes were shown in Figure 5.3. The glowing wire in the figure demonstrates the successful attachment of λ-DNA between two gold electrodes. Several repeatable data points were obtained; but this particular case of attachment corresponds to 5ng/µL concentration of λ-DNA. The area between the electrodes is approximately 8µm~20µm. At the stated fixed resolution, microscope photos of the electrode substrate, midline, and surface were obtained which demonstrates the depth of the electrode features (~75µm). The different levels of focus demonstrate how the DNA is suspended between the top surfaces of the two gold electrodes and not in contact with the silicon substrate.

![Figure 5.3. DNA attachment Images taken by varying the focus of fluorescent microscope: (a) focused on electrode, (b) on substrate, (c) on the mid-plane where DNA mostly visualized.](image)

This is one of the key objectives of this research where it was desired to keep the DNA as far away as possible from the substrate to eliminate the detrimental effect of the substrate. The images where the focus was on the electrodes and reduced illumination in midline plane exhibit the highest fluorescence suggesting that these areas most likely attract oligos and DNA. Figure 4 shows edges of the microelectrodes where there is significant concentration of attached oligos and folded DNA.

Following are the microscope settings used for our visualization:
- Resolution: W: 1392, H: 1040
- Final preview resolution: 696 × 520
- Set area: (0, 0) - (1391, 1039)
- Camera exposure upper limit: 500 ms
5.1.2 Visual Image from Scanning Electron Microscopy (SEM)

The SEM is a type of electron microscope that images the sample surface by scanning with a high-energy beam of electrons in a raster scan pattern [88]. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample’s surface topography, composition and other properties such as electrical conductivity.

Figure 5.4. SEM image of DNA molecular wires
5.1.3 High Frequency AC Impedance

Electrical resistance measurements were performed on the microelectrode structure where λ-DNA was successfully attached. The characterization was done in a dry environment after washing the chip with DI water. Typically about thirty minutes to an hour were needed before stable measurement can be recorded. Further, the experimental condition consisted of strictly environment inside UV-protected location at room temperature. The characterization consisted of I-V curve under DC current and AC impedance analysis using Solartron Analytical Model 1070E (manufactured by AMETEK advanced Measurement Technology, Oakridge, TN). This consisted of linear sweep voltametry from -2 to 2 Volt at a scan rate of 100 mV/s and Galvanostatic impedance measurement in frequency range from 1 MHz to 1 Hz.

Electrical conductivity of DNA wire was confirmed from the DC linear sweep voltametry and the result was plotted as an I-V curve as shown in figure 5.4 below.

![Figure 5.5. Linear sweep voltametry result of attached DNA to the gold electrodes](image)

Impedance analysis was done with a frequency sweep from 1 MHz to 1Hz and the results were plotted as a standard Bode plot (impedance and phase versus frequency) (figure 5.5).
The Bode plot shown in figure 5.5 suggest that - at low frequencies - the λ-DNA - high aspect-ratio microelectrode structures tends to exhibit impedance properties similar to metals where the impedance is constant (at about 2MΩ) and the phase is almost zero. This impedance at lower frequencies is - as expected - similar to the direct electrical resistance measurements obtained with DC current using four-point probe. At around 100 Hz frequency, however, the phase is no longer negligible (indicating the presence of an imaginary component) and the impedance begins to drop sharply with steep shifts in phase. At 1 MHz frequency, the impedance of the λ-DNA molecular wire goes down to less than 10 KΩ. This is a significant finding indicating the rather unexpected behavior of this molecular wire at high frequencies.

Figure 5.6. AC impedance results for DNA molecular wires suspended between high aspect-ratio gold electrodes
As a comparison, the impedance curve of a gold thin-film wire is given in the same chart with characteristics straight-line in low as well as high frequencies.

### 5.1.4 Electron Dispersion Spectroscopy (EDS) Result

The next experiment was done to answer the question whether it is DNA who is transferring the electrical signal from one electrode end another, or is it something else. For this purpose, Electron Dispersion X-ray Spectroscopy (EDS) was used for the material characterization. EDS uses silicon drift detectors (SDD) and integrative software systems to determine the elements in a material by bombarding the material with X-rays, allowing the material to absorb the X-rays which thus becomes a charge that strikes the detector and is converted to a voltage signal through a preamplifier [89]. The amount of X-ray absorbed is dependent on the element and since the output voltage is directly proportional to the amount of X-ray absorbed [88], the elements in each sample can easily be quantified.

Figure 5.7. EDS result of the chip with DNA bridge. DNA concentration is 0.25ng/µl
EDS was done for the chip that has DNA molecular wire in three spectrums along the x-axis. As we can see in figure 5.5 above, spectrum one and three were done on the top of each electrodes and it recognized that gold (Au) was one of the element existed on the surface of the electrode (in a red circle). The spectrum analysis on the gap, the location where we suspect that the DNA exist as connecting wires shown different result compares to the other two spectrums. The non-existence of gold at that location was a good indication for the electrode to really have a gap in between them, while the quantitative result of the material analysis shown that materials existed in that location were all organic materials coming like Carbon (C), Nitrogen (N), Oxygen (O), Sulfur (S) and Phosphorous (P) came from the DNA and Chlorine (Cl) and Sodium (Na) came from the DNA buffer.

EDS was also further utilized to map the location in where each material existed. The result from this mapping was aligning with the previous spectrums reading.

Figure 5.8. Element mapping of DNA chip, concentration 0.25ng/µl. Top row are for the control chip and bottom row are for the DNA chips. Element shown are Carbon and Chlor

Figure 5.6 above shows that Carbon that was earlier existed mostly on the electrode (as one of the main material of SU-8 photoresist); get a significant addition mostly on the edge of the
electrodes as the indication of oligo and DNA attached to the electrodes. In similar manner, Chlorine was also getting significant addition on the same location as the element is one of the main components of the buffer.

Figure 5.9. Element mapping of DNA chip, concentration 0.25 ng/µl. Top rows are for the control chip and bottom row are for the DNA chips. Element shown are Oxygen and Phosphorous

Align with figure 5.6, we also observe similar addition for oxygen and phosphorous after attachment as shown in figure 5.7 above.

5.2 Optimizing Concentration of DNA Wire

After encouraging result of the DNA attachment, the experiment was brought one step further by conducting experiments with various DNA concentrations. One key factor that was investigated with this research was the optimum concentration of DNA that would result in DNA molecular wire bridge that contains as fewer as DNA strands as possible between the electrodes. In the ideal condition, a bridge that contains a single DNA wire was
sought. This experiment was done by varying the concentration of DNA from the original concentration of 500 ng/µl all the way down to 0.0625 ng/µl. For detection of a molecular wire bridge, both optical detection through fluorescent tagging and electrical detection by running a voltametry and AC impedance were used.

![Figure 5.10. Image of DNA molecular wire in various concentrations](image)

The first set of result involved determining an optimum concentration of DNA that would result in a DNA molecular wire bridge that contains fewer DNA strands as possible between the electrodes. For optical detection using fluorescent tagging, the limiting concentration below which no detection was possible was determined to be 1 ng/µl as summarized in figure 5.8. Based on a simple calculations, this concentration corresponds to as small as a single DNA molecular wire (the volume considered near attachment area = 75 µm x 8 µm x 20 µm = 12,000 µm². Therefore total weight = 1.5 x 10⁻⁶ ng. 1 µm has 0.03 pmols or 2.1x10¹⁰ molecules. 1 ng of it has 2.1 x 10⁷ molecules; Therefore 1.5x10⁻⁶ ng~≈3 molecules (lower bound - assuming 10% attachment).

**ImageJ** software was then used to measure and compare number of pixels in raw images without any image processing. Average of the 6 images was measured along with
standard deviation. The mean of the measured intensities of the images was used for the comparison and analysis. The analysis command “Measure” of ImageJ was used. “Measure” calculates and displays area statistics along a line length or a region. Within same sized area selections, the mean, standard deviation, mode, min and max parameters were recorded. The mean and standard deviation were calculated from the values of the pixels in the area.

Figure 5.9 summarizes the result where the first y-axis represents impedance ($|z|$) and the second represent fluorescent intensity. It is interesting to note that with the decrease in the number of DNA molecular wires covalently attached to the electrodes, the impedance was initially decrease but at some point it started to increase up to what is perhaps a value corresponding to that a single DNA molecular wire. These trends correspond to a logic where the addition of DNA wires will increase the conductivity as we have more wire means more paths to conduct the electric charges, but at some point all the possible location for DNA to attached has been occupied and the addition of wires will just create three-dimensional tangling between the wire and will start reducing the conductivity.

![Figure 5.11. Optimization of DNA concentration based on electrical conductivity and fluorescent intensity.](image-url)
Analysis based on Finite Element Modeling (FEM), as shown in Figure 5.10, of the platform offers a supporting argument for the decrease in impedance with the increase number of molecular wires spanning between the two electrodes in any arbitrary position.

![Concentration Vs Resistance (FEA)](image)

Figure 5.12. Finite Element Modeling (FEM) of the DNA molecular wire-based platform (Courtesy of Varsha Ramesh)

### 5.3 EFFECT OF TEMPERATURE TO DNA MOLECULAR WIRE

The effect of temperature to the DNA molecular wire is one of the main research objectives in this thesis. This study is very important in explaining many factors related to this experiment such as DNA denaturation, bond dissociation, electrics capacitance and DNA conductivity. More important, we can use this as a tool to prove that the wire is indeed made of DNA and we can use it also in supporting the theory of charge transport mechanism.

#### 5.3.1 Control Experiment

The first control in the experiment is the environment control and for this purpose the chip that is contained DNA molecular wire was placed in a box made of carbon composite hosing and kept in a refrigerator overnight at 4°C with enough amounts of desiccants to keep the wire dry. This chip was then purged with N\textsubscript{2} and the subsequently during the experiment when it subjected to temperature increment of 5°C on a hot plate. At each temperature of
interest, two minutes of stabilization were followed by AC impedance and voltametry measurements.

The second control experiment has purpose to eliminate the possibility of energy accumulation that affect the DNA wire conductivity. For this purpose, first chips with DNA molecular wire were heated up to 40°C and impedance measurements were done in the interval of 2 minutes for 20 minutes measurement. The fact that impedance measured in the tolerable range during the 10 data points show that the electrical conductivity of DNA wire is a function of temperature and not energy accumulation. Another control that is done in the same purpose is the measure the impedance in two different ways. The first is by ramping it up from the cold condition up to denaturation and the second is by heating it up to 75°C (below the melting temperature so that the denaturation is still reversible) and measure the impedance during cooling down every 5°C down.

The third control experiment was done to prove that it was indeed DNA molecular wire that transfers the electricity and not some other material. In this experiment, we did similar experiment by measuring impedance every 5°C interval for four different chips. First chip is a mithras electrode with no gap (gold layer connecting the electrode), second chip is a mithras electrode with gap but no DNA attached to show that we were not measuring the capacitance between the gap, third chip is mithras electrode with DNA solution but not having any attachment and the fourth chip is the mithras electrode with attached DNA molecular wire. From this control experiments, we can see the difference of impedance in effect of temperature from our DNA molecular wire (chip #4), gold electrode (chip #1), DNA buffer solution (Chip #3) and an open circuit (chip #2).

5.3.2 Experiment Result – Temperature Effects

This experiment was done until DNA completely denatures at its melting point and the indication is the open circuit during impedance measurement. Figure 5.10 gives the I-V curve and impedance measurements are shown in figure 5.11.
As expected, temperature had a significant effect on the impedance of DNA molecular wire. However the effects are kind of peculiar and involves what appears to be a
two-stage effect involving lower temperatures (4°C - 40°C) and moderately higher temperatures (40°C - melting point). This result is shown more clearly in the plot of temperature-impedance at various frequencies as shown in figure 5.12 below.

![Temp. VS Impedance @ 1 kHz](image1)

![Temp. VS Impedance @ 10 kHz](image2)

Figure 5.15. Variation of AC Impedance of DNA molecular wire at different temperature. DNA concentration = 0.25 ng/µl.

In the first range of temperatures, the AC impedance increase until it reaches about 40°C, a temperature which denaturation is started to occur. This followed by a decrease in impedance all the way to the melting point which result in complete denaturation of λ-DNA wherein
conductivity is lost. Leaving the denatured DNA molecular wire to cool down will not bring the DNA wire back to the initial conductivity. A hysteresis like behavior (as in Figure 5.13) were shown by the DNA wire as the electrical conductivity measurement shown different path with a tendency of the wire become less conductive compare to the conductivity before denaturation.

Figure 5.16. Hysteresis behavior shown by DNA molecular wire. DNA concentration = 0.25 ng/µl
Subsequent renaturation of the λ-DNA though incubation in a TM buffer (a combination of Tris-EDTA and Sodium Chloride at pH 7) solution followed by temperature recycling (to go up to 10°C below the melting point) resulted in recovery of current conduction, providing a strong proof of the presence of a conducting DNA molecular wire bridge. Addition of Sodium Chloride in the TM buffer is mainly to control the pH with an acid that is similar with the acid contained in the original EDTA buffer, while adding some proton might also be necessary if we need to increase the pH level. In some study, histidine is one of the widely used as the source of proton [90].

![Figure 5.17. Impedance curves of DNA molecular wire at room temperature, at some temperature during denaturation, and after renaturation process. DNA concentration = 0.25 ng/µl.](image-url)
CHAPTER 6

DISCUSSION

In this chapter, we will critically examine our results both from the mechanics and biochemistry of attachment and electrical properties point of view. The first part of the discussion will be focusing on the optimization of DNA attachment process which includes both effectiveness of the attachment method and electrical conduction-based optimum concentration. Second part will discuss in detail the charge transport mechanism and how the result of temperature effect experiment can further support the theory.

6.1 OPTIMIZING DNA ATTACHMENT

The oligo-mediated DNA attachment to microelectrode structures is complex effort that required optimization at different level in the biophysics and chemistry of the attachment, engineering of the microelectrode structure and the mechanics of the delivery system including the utilization of microfluidics. The final considerations of optimum condition are the high yield of DNA attachment process and optimum λ-DNA concentration to produce DNA molecular wires with highest electrical conductivity.

6.1.1 Optimum Attachment Method

From the engineering of DNA attachment perspective, one of the key challenges was how to deliver the solutions contains DNA as close as possible to the electrode gap. In our approach, this was done through manual pipetting; but further refinement through microfluidics approach continues and applying electrical bias was also proven to be an effective method to attract the DNA wire closer to the electrodes. Another related issue is the need to promote optimum ligation of DNA and oligos, and having the ligation process on the chip (instead of in the tube) was proven to be the optimum method. Oligo attachment to the electrodes before its ligation to the λ-DNA was done by having a relatively high concentration of oligos, and the goal this approach is to populate most of the electrodes surface with oligo which means increase of the number of potential binding site for the λ-DNA. In this case, 1μL of 1μM oligo concentrations was dropped to populate the electrodes
surface and followed by ligation process which include the addition of 1μL of DNA ligase mix and 1μL of λ-DNA in various concentration were used with the highest concentration was the initial λ-DNA solution at 500 ng/µl.

Applying low voltage electrical bias during the attachment process was also proven to increase the chance of getting DNA attachment. The main reason is that DNA has negative charge that will be attracted to the positive charge electrode when a bias applied. The electrical bias need to be controlled in a low voltage and the application should not be more than five times since the conductive part of the electrode is only the thin 6 nm gold layer on the surface of the electrodes. Applying too high voltage or repeating too many times will cause the gold layer to flake and the electrical conductivity measurement will not be accurate anymore.

6.1.2 Optimum Concentration to Optimize AC Electrical Conductivity

One of the finding of this research that may have significant implications to the field of DNA-based bionanoelectronics could be the low impedance of λ-DNA molecular wires observed at moderate to high frequencies. While the overwhelming majority of previous research work in this area had concentrated on direct resistance measurement under DC conditions, these new findings offer new incentives to further investigate electrical property of DNA molecular wires at high frequency AC current. For now, we believe the explanations for this rather low impedance at high frequencies may be found from the theoretical models of current transduction in DNA based on charge hopping in π-stacked base pairs if one assumes that - at high frequencies - tunneling across base pairs may be the dominant mode.

In regards to the electrical conductivity of DNA molecular wire, variation in λ-DNA concentration has resulting in different electrical conductivity. As shown in Figure 5.9, the first part when the DNA concentration increased, the electrical impedance decreased (conductivity increased). The logic behind this circumstance is that as we add the number of DNA molecular wire, we will have more paths to transport the electrical charge from one side to the other. But at some point, DNA wires will occupy all of the possible location, and the addition of DNA wire will not increase the conductivity but instead decrease the conductivity as the additional wires will be tangled and even creating DNA clumps that can longer the path of DNA charge transport. This result is confirmed by the similar result
produced from the finite element analysis of the DNA molecular wire as shown in Figure 5.10.

6.2 CHARGE TRANSPORT MECHANISM ALONG DNA WIRE

Before we take up issues concerning the charge migration within the DNA duplex, it is instructive to examine the necessary basic principles pertaining to the long range electron transfer processes. The electrons of different types of atoms have different degrees of freedom to move around. These virtually unbound electrons are free to leave their respective atoms and float around in the space between adjacent atoms. They are often called free electrons. This relative mobility of electrons within a material is known as electric conductivity. No material is fully an insulator, in other word, perfect insulator does not exist. Materials always have some degree of conductivity [91]. Most insulators have a large band gap. This occurs because the "valence" band containing the highest energy electrons is full, and a large energy gap separates this band from the next band above it. But there is always some voltage (called the breakdown voltage) that gives electrons enough energy to be excited into this band. Once this voltage is exceeded the material ceases being an insulator, and charge begins to pass through it. In relation with temperature: normally insulating material, also turn to be conductive if heated to very high temperatures because the thermal energy will also excite electrons and move to higher energy band. But in opposite, most metals become poorer conductors when heated, and better conductors when cooled [91].

6.2.1 Quantum Physics of Electron Transport

The easiest way to describe how quantum mechanics is different from the traditional physics is described in figure 6.1. A more general situation can be described as particle incident on a “potential energy barrier”. In Classical Mechanics, particle will approach potential maximum until it reaches the turning point, just like a ball rolling up a hill. When the action potential can overcome the potential barrier then the ball can travel to the other side of the hill, on the other hand when the action potential is lower than the barrier then it will not reach the other side and bounce back to the initial condition. Quantum mechanics observe an effect known as quantum tunneling where particles incident on such a barrier have a nonzero probability of being found on the other side [92].
Tunneling is a quantum-mechanical effect, essentially based on the overlap of electronic wave functions: Electrons are able to "tunnel" across a gap between two electrodes, provided this gap is small enough (on the order of a few nanometers). The larger the electronic overlap, the higher the tunneling probability. In theory, electron tunneling is possible to occur in any molecules, or probable to happen. In order for the probability of tunneling to be significant, potential barrier heights and length scales must be small. This condition restricts the classes of systems that may exhibit significant tunneling. This idea of quantum tunneling has opened a new window in the discussion of a charge transport through a molecule, in particular along DNA molecular wire. Adopting the quantum tunneling in our experiment, Figure 6.2 explains how the probability of electron transport through the electrodes is highly possible through tunneling.

6.2.2 Quantum Tunneling in DNA wire

Earlier studies in electron tunneling along DNA molecules has constituted that tunneling occur through the \( \pi \)-stacking of the phosphate backbone \([20, 93-96]\). Quantum tunneling a natural process in biology, it happens almost in all metabolism processes like photosynthesis, oxidative phosphorylation and all other processes that involving energy transport \([93]\). Based on this idea, charge tunneling through DNA molecule should be
something natural. Since quantum tunneling probability is based on a distant between donor and acceptor, with this understanding we adopt the phenomena to the DNA wire. Figure 6.3 shows how the distance between our two electrodes are not 12 µm but a lot smaller than that.

Figure 6.2. Illustration of how electron transport through the electrodes that has low possibility based on classical mechanics (left figures) become highly probable in the context of quantum mechanics (right figures)

Figure 6.3. Illustration on breaking down the distance from 10 µm gap to be a very small distance between two atoms in the DNA phosphate backbone.
Further breaking down of the phosphate backbone is illustrated in figure 6.4 below. From figure 6.4 we can see that inside the long phosphate backbone of dsDNA, beside the aromatic ring and hydrogen bond, there are only three different bonds that connect the chains, which are bonds between O-P, O-C, and C-C. From the chemistry of bonding, in a covalent bonding where each atom will share their outer electron, the maximum bond distance is going to be the addition of both atomic radiiuses [97]. It supported by the data from the bond length database [97], the bond distance between O-P = 1.38 Å, O-C = 1.24 Å, C-C = 1.51 Å, while the atomic radius of O = 0.66 Å, C=0.76 Å, P = 1.07 Å [98]. In some different molecules, a same covalent bond might have slightly different bond distance because the three-dimensional location of each atom might create some angles between the two atoms.

Figure 6.4. Detail Primary Structure of the Watson and Crick's DNA phosphate backbone [100]
Lewis et al. in the proceeding of presenting an experiment in distant dependent charge tunneling in DNA [99] concludes that in a single step tunneling in DNA, electron transport can proceed upon a distance ~ 2.7 Å. This conclusion means that tunneling can occur through each bond in DNA phosphate backbones; make a long distance charge transport in a long DNA wire possible.

6.2.3 Charge Hopping in DNA Wire

The second widely discussed charge transport mechanism in DNA is charge/hole hopping [102]. Most of the earlier researches in this area conclude that the charge hopping in DNA molecules occur between hydrogen bond in its G:C base pairs [101-103]. The quantum physics make it possible for the charge to travel from one sub-equilibrium state to another by means of hopping through the potential barrier, achieving this "quantum jump" from one state to another [101]. In this case the charge is not moving through the backbone or DNA connecting bonds (phosphate backbone), but it is hopping through the lowest energy gap that exists in hydrogen bond. It explained why single strand DNA (ssDNA) is not conductive in some degree since the phosphate backbone energy gap is still too high for a charge to hop through. They can only rely in tunneling which is effective only in a very low temperature [29].

![Figure 6.5. Illustration of charge hopping through hydrogen bond in the G:C pairs along the DNA molecular wire [102]](image-url)
This charge hopping through hydrogen bond can be understood with reason that hydrogen bond possess the lowest energy potential compare to other bond that exist in DNA strand [104-105]. Due to the asymmetry of energy in DNA it is then assumed that the charge will occupy the potential well of lowest energy, therefore charge/hole would like to occupy this energy well.

### 6.2.4 Bond Dissociation

Understanding the concept of bond dissociation energy is very important in the study of DNA molecular wire. Having many bonds exist in the DNA molecular wire platform, understanding the thermodynamics of each bond is very critical, especially when we discuss how the external energy will affect the structure of DNA molecular wire. In this experiment, we will use the concept of bond dissociation energy to understand which bond is going to dissociate before the others when applied to temperature increment.

The bond dissociation energy ($\Delta H_{\text{dis}}$) for a bond $A-B$ which is broken through a reaction

$$AB \rightarrow A + B$$

is defined as here as standard state enthalpy change for the reaction at a specific temperature. That is,

$$\Delta H_{\text{dis}} = \Delta H_f(A) + \Delta H_f(B) - \Delta H_f(AB)$$

where $\Delta H_f$ is standard state energy of formation [104].

### Table 6. Bond Dissociation Energy of Molecules in DNA [104]

<table>
<thead>
<tr>
<th>Bond Location</th>
<th>Bond Name</th>
<th>Molecules after Dissociation</th>
<th>Approx. MW (g/mol)</th>
<th>Dissociation Energy ($\Delta H_{\text{dis}}$) at 298 K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrode - Oligo</td>
<td>Au-S</td>
<td>Au + SH</td>
<td>230.05</td>
<td>253.6</td>
</tr>
<tr>
<td>Oligonucleotide</td>
<td>S-O</td>
<td>SH + PO$_4$</td>
<td>128.05</td>
<td>124.69</td>
</tr>
<tr>
<td>Phosphate Backbone</td>
<td>O-P</td>
<td>P &amp; 2 of O$_2$</td>
<td>94.97</td>
<td>142.3</td>
</tr>
<tr>
<td></td>
<td>C-O</td>
<td>Ribose + PO$_4$</td>
<td>245.1</td>
<td>256.89</td>
</tr>
<tr>
<td></td>
<td>C-C</td>
<td>Ribose + CH$_3$</td>
<td>165.18</td>
<td>144.2</td>
</tr>
<tr>
<td>Sugar Base pairs</td>
<td>H-H</td>
<td>Guanine + Cytosine</td>
<td>262.28</td>
<td><strong>104.19</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adenine + Thymine</td>
<td>261.23</td>
<td><strong>104.19</strong></td>
</tr>
</tbody>
</table>
Using bond dissociation energy data as shown in Table 6, we can see that hydrogen bond possesses the lowest dissociation energy compared to other bonds exist in the DNA wire as shown in Figure 6.3 earlier. We can further estimate the temperature to dissociation based on the understanding on equation 3 below.

\[ \Delta H_{dis} = \Delta nRT \]  

(3)

Based on the molecular weight (MW) data as in table 4 below, the estimate temperature to dissociate each bond is presented in table 7.

**Table 7. Molecular Weight Data of Molecules in DNA Molecular Wire [105]**

<table>
<thead>
<tr>
<th>Atoms</th>
<th>MW (g/mole)</th>
<th>Molecules</th>
<th>MW (g/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>1.01</td>
<td>SH</td>
<td>33.08</td>
</tr>
<tr>
<td>C</td>
<td>12.01</td>
<td>PO₄</td>
<td>94.97</td>
</tr>
<tr>
<td>O</td>
<td>16</td>
<td>CH₃</td>
<td>15.05</td>
</tr>
<tr>
<td>P</td>
<td>30.97</td>
<td>Ribose Sugar</td>
<td>150.13</td>
</tr>
<tr>
<td>S</td>
<td>32.07</td>
<td>Thymine</td>
<td>126.113</td>
</tr>
<tr>
<td>Au</td>
<td>196.97</td>
<td>Adenine</td>
<td>135.13</td>
</tr>
<tr>
<td>N</td>
<td>14</td>
<td>Cytosine</td>
<td>111.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Guanine</td>
<td>151.13</td>
</tr>
</tbody>
</table>

**Table 8. Calculation Summary of Bond Dissociation Temperature**

<table>
<thead>
<tr>
<th>Bond Location</th>
<th>Bond Name</th>
<th>Molecules after Dissociation</th>
<th>Approx. MW (g/mole)</th>
<th>Dissociation Energy ((\Delta H_{dis})) at 298 K, kJ/mole</th>
<th>Dissociation Temperature (K)</th>
<th>Dissociation Temperature (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrode - Oligo</td>
<td>Au-S</td>
<td>Au + SH</td>
<td>230.05</td>
<td>253.6</td>
<td>441.974</td>
<td>168.97</td>
</tr>
<tr>
<td>Oligonucleotide</td>
<td>S-O</td>
<td>SH + PO₄</td>
<td>128.05</td>
<td>124.69</td>
<td>390.40</td>
<td>117.41</td>
</tr>
<tr>
<td>Phosphate Backbone</td>
<td>O-P</td>
<td>P &amp; 2 of O₂</td>
<td>94.97</td>
<td>142.3</td>
<td>450.55</td>
<td>177.56</td>
</tr>
<tr>
<td></td>
<td>C-O</td>
<td>Ribose + PO₄</td>
<td>245.1</td>
<td>256.89</td>
<td>420.21</td>
<td>147.22</td>
</tr>
<tr>
<td></td>
<td>C-C</td>
<td>Ribose + CH₃</td>
<td>165.18</td>
<td>144.2</td>
<td>420.00</td>
<td>147.01</td>
</tr>
<tr>
<td>Sugar Base pairs</td>
<td>H-H</td>
<td>Guanine + Cytosine</td>
<td>262.28</td>
<td>104.19</td>
<td>318.53</td>
<td>45.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adenine + Thymine</td>
<td>261.23</td>
<td>104.19</td>
<td>319.81</td>
<td>46.82</td>
</tr>
</tbody>
</table>
The result is just an approximation because the calculation is based on the assumption that each bond is an independent molecule and not just a part of longer DNA strand. The result above is summarizing in a simple figure as shown in Figure 6.6 below.

Figure 6.6 Illustration of bond dissociation energy exist in a DNA molecular wire [105]

6.2.5 Measuring Conductivity of DNA Wire

One of the reported techniques used to measure the intrinsic conductivity of DNA is resonant cavity. It uses a configuration that does not require contacts to be attached to the sample under study [106]. A resonant cavity encloses a volume whose dimensions are comparable to the desired wavelength and can support a series of modes each corresponding to a unique distribution of field. Most resonators are fabricated out of highly conducting materials so that the field is determined only by the boundary conditions at the inner surfaces. In a perfect cavity, the energy loss is zero and the quality factor infinite. In reality, the surface of the cavity has some finite impedance and the fields penetrate the walls as the effective shielding by the induced currents is reduced [109].

Dry DNA, DNA in buffer solution and buffer alone are measured. The results are shown in Figure 6.7. The conductivity follows an exponential law of disordered system [111]
\[ \sigma = \sigma_0 \exp(-\Delta/2kT) \] (4)

Figure 6.7. Conductivity of different \( \lambda \)-DNA at different frequencies as function of inverse temperature. The lines follow the law given by Eq. 6.1 [106]

DNA is also measured in microwave cells (i.e. without contact to it). The conductivity is deduced from the decrease of the microwave power reflected by the cell. The difference with Ref. [111] is that the charges are induced with pulses of 3 MeV electrons from a Van De Graaf accelerator, so the conductivity depends on the electron dose. Measurements are done at a fixed temperature (195 K) and for different degrees of DNA hydration. The conclusion is that the conductivity in B-DNA is due to mobile charge carriers within the outer mantle of the chain rather than within the base-pairs cores (notably due to the absence of anisotropy of conductivity for aligned fibers). There is however no explanation why these electrons do not become rapidly localized within the DNA core [113].
6.2.6 Temperature Effect to Charge Transport in Semiconductors

The conductivity in semiconductors is typically in the range of $10^{-9} - 10^{3} \text{ S cm}^{-1}$, respectively ($S = \text{Siemens or W}^{-1}$) [91]. The conductivity of metals decreases when increasing of temperature applied, but in opposite for semiconductors as it increases. Such aspects are usually explained by the band theory of solids. Just as atomic orbitals combine to form molecular orbitals in molecule, they combine to form a very large number of states. The energy levels corresponding to the states formed from a given type of orbital can be treated as a continuous band of energy. Since the atomic orbitals are discrete levels with energy gaps in between, the energy bands formed from the different atomic orbitals leave regions of energy in between where the entry of electrons is forbidden.

![Figure 6.8. Illustration of Silicon semiconductivity based on the band theory](image)

Packing of available electrons from the lowest energy upwards, leads to the final band containing electrons to be partially filled; the highest level occupied by electrons is called the Fermi level. This allows inactivated electron transport and is a characteristic of metals.

When metals are heated the resistance increases because of increased lattice vibrations which impede electron flow. Packing of available electrons from the lowest energy upwards in a band structure can also end up with a completely filled band (valence band) and a higher energy empty band (conduction band), the two being separated by an energy gap. This situation leads to thermally activated conduction, characteristic of semiconductors. Heating causes increased lattice vibrations in semiconductors as it simultaneously leads to higher population of charge carriers in the conduction band and hence increase in conductivity.
Conductivity of a material is determined by two factors: the concentration of free carriers available to conduct current and their mobility (or freedom to move). In a semiconductor, both mobility and carrier concentration are temperature dependent. There are two basic types of scattering mechanisms that influence the mobility of electrons and holes: lattice scattering and impurity scattering. We have already discussed lattice scattering in the context of metals; we know that lattice vibrations cause the mobility to decrease with increasing temperature [108].

![Figure 6.9. Approximate temperature dependence of mobility with both lattice and impurity scattering [108]](image)

However, the mobility of the carriers in a semiconductor is also influenced by the presence of charged impurities [108]. Impurity scattering is caused by crystal defects such as ionized impurities. At lower temperatures, carriers move more slowly, so there is more time for them to interact with charged impurities. As a result, as the temperature decreases, impurity scattering increases and the mobility decreases. This is just the opposite of the effect of lattice scattering. Impurity scattering is typically only seen at very low temperatures. In the temperature range we will measure, only the influence of lattice scattering will be expected. The temperature effect to the charge mobility shown in Figure 6.9 is supporting our experiment result in the effect of temperature as shown earlier in Figure 5.12. With charge mobility supporting electrical conductivity, as shown in figure 5.12, our result showed the same two temperature regions that define the response of electrical conductivity to the
change in temperature. This result once again supporting the idea that DNA molecular wire behaves as a semiconductor material.

6.2.7 DNA Molecular Wire as an Organic Semiconductor

Organic semiconductors can be broadly classified into two categories: small molecules or oligomers (usually processed in vacuum) and polymers (usually processed by wet chemical techniques) [112]. In each case, various materials have been designed over the years that preferentially transport holes or electrons. In most instances, this distinction does not rely on the actual ability of the materials to transport charges (i.e., on the actual values of charge mobility) but rather reflects the ease of charge injection from electrodes traditionally used in devices. In that context, a material is often referred to as a hole [electron] transporter when its ionization energy [electron affinity] closely matches the Fermi level of the electrode material. Ambipolar transport (i.e., the ability to transport both electrons and holes) has now been reported for several organic semiconductors [112].

Efficient charge transport requires that the charges be able to move from molecule to molecule and not be trapped or scattered. Therefore, charge carriers mobility are influenced by many factors including molecular packing, disorder, presence of impurities, temperature, electric field, charge-carrier density, size/molecular weight, and pressure. It would be too formidable a task to try to discuss all the experimental studies reported to date on the impact of these parameters on charge transport in organic semiconductors.

In the absence of chemical and physical defects, the nature of charge transport depends on a subtle interplay between electronic and electron vibration (phonon) interactions. In the case of the traditional, covalently bound inorganic semiconductors, the electron-phonon interactions are usually much smaller than the electronic interactions and simply account for the scattering of highly delocalized carriers. In contrast, in organic (macro) molecular semiconductors, the extensive experimental and theoretical investigations of the last decades have shown that the electron-phonon interactions are comparable to, or even larger than the electronic interactions (we recall that a phonon is a particle-like quantized mode of vibrational energy arising from the collective oscillations of atoms within a crystal). In such a case, electron-phonon coupling no longer plays the role of a perturbation
but rather leads to the formation of quasiparticles, polarons, in which the electronic charge is
dressed by phonon clouds [107].

Same as in inorganic semiconductor, temperature raise will generally also induce two
opposite phenomena in relation to electrical conductivity. For once it will introduce external
energy that can excite electron (hence increase electrical conductivity via hopping) but in the
other hand it also promote lattice vibration and molecular expansion that reduce the electrical
conductivity via tunneling. Even in organic semiconductor it is getting more complication
with the electron-phonon coupling and polaron formation.

**Figure 6.10.** Temperature dependence of the charge mobility predicted by
Holstein polaron model for the limiting cases of strong and weak
electron-phonon couplings [107].

Here, the first term is due to electron tunneling (coherent electron transfer) and dominates
transport at low temperatures; the second term is related to hopping motion (incoherent
electron transfer) and becomes dominant at higher temperatures. The relative contributions of
each mechanism depend, however, on the actual values of the microscopic parameters
(electron-phonon coupling, electronic and phonon bandwidths, and phonon energy).
Illustrative examples of the temperature dependence of the mobility for large ($g^2 >> 1$) and
weak \( g^2 << 1 \) electron-phonon couplings are schematically depicted in Figure 6.10. In the case of weak local electron-phonon couplings \( (g^2 << 1) \), the mobility is dominated by tunneling and displays a band-like temperature dependence \( (u\sim T^n, \text{ where } n > 0) \) in the whole range of temperatures. For intermediate \( (g^2 \leq 1) \) couplings, the mobility is band-like at low temperatures; however, due to a significant increase in hopping contributions, it exhibits a weaker temperature dependence at high temperatures. For strong local couplings \( (g^2 >> 1) \), three distinct temperature regimes occur; see Figure 6.10: (i) at low temperatures \( (T < T_1) \), the mobility is band-like; (ii) as temperature increases, the hopping term starts to dominate, and the mobility exhibits a crossover from coherent transport to an incoherent, temperature-activated transport; (iii) if the system can reach very high temperatures \( (T > T_2) \) at which the thermal energy becomes large enough to dissociate the polaron, the residual electron is scattered by thermal phonons and as a result the mobility decreases again with temperature. The crossover temperatures \( T_1 \) and \( T_2 \) are defined by the combination of microscopic parameters; depending on the actual values of \( T_1 \) and \( T_2 \), only one or two transport regimes might be experimentally accessible for a particular system.

Again the theory (especially the strong electron phonon coupling) is very much aligns with our experiment result as shown in figure 5.12. The differing impedance report at two temperature regimes in our experiment result: impedance decreases between \( 4^\circ C - 40^\circ C \) and then decreases between \( 40^\circ C - \text{melting point (~110}^\circ C\)) after which \( \lambda \)-DNA denatures resulting in no current transduction. This means that \( \lambda \)-DNA shows similar behavior with organic semiconductor as far as effect of temperature is concerned.
CHAPTER 7

CONCLUSION

In this study, we had investigated the attachment to high aspect-ratio electrodes and DC and AC electrical characterization of double-stranded DNA (λ-DNA) as well as the effect of temperature on their electrical conductivity. The attachment of DNA to metal electrodes was done through self-assembly of thiolated oligonucleotides (at the DNA ends) and covalent bonding between DNA and gold electrodes. We had used both negative and positive controls in the experiments.

Based on the evidence presented in this study, the following conclusions are made:

1. The attachment of DNA to microelectrode structures through oligo-mediation is optimized using the ligation of phosphate backbone, resulting in much higher yield of attachment compare to the hybridization method.
2. DC direct electrical resistance measurements based on several data points of strongly suggest that λ-DNA has a measurable and significant conductivity (Resistance of 2MΩ - 25MΩ) that could establish it as a semi-conductor.
3. AC impedance measurements strongly match DC direct resistance measurements at lower frequencies where the impedance mainly consists of Ohmic resistance.
4. At higher frequencies, the impedance of λ-DNA molecular wire appears to be significantly low - almost to the level of good metallic conductors. This could possibly be explained by an enhanced tunneling electron transport in π-stacked base pairs.
5. Detailed temperature dependence experiments that traced the impedance response of λ-DNA till full denaturation helped establish that it is indeed λ-DNA molecular wire that is generating this sort of AC conductivity.
6. Subsequent renaturation of the λ-DNA through incubation in a buffer solution followed by temperature recycling resulted in recovery of current condition providing a strong proof of the presence of a conducting DNA molecular wires bridge.
7. Further, these experiments on the effects of temperature on impedance response shed a light on conductivity mechanism along DNA molecular wires. At high temperatures, the expected increase in charge hopping mechanism may account in the decrease of impedance (and hence increase in conductivity) supporting the charge hopping mechanism theory.

8. We also report that λ-DNA molecular wires have differing impedance report at two temperature regimes: impedance decreases between 4^0^C - 40^0^C and then decreases between 40^0^C - melting point (~110^0^C), after which λ-DNA denatures resulting in no current transduction. This means that λ-DNA shows similar behavior with organic semiconductor on the effect of temperature.

9. The two different responses in two temperature regimes mean that λ-DNA has a potential to be used as a temperature switch.

10. The fact that hydrogen bond has the lowest dissociation energy explained the reason of electrical charge occupy the bond as the well of potential energy, hence promote hopping through these hydrogen bonds.

11. Calculation of temperature to dissociation for each bond existed in the bionanoelectronics platforms, shows that hydrogen bond between the base-pairs will be the first to dissociate before others. This support the idea that temperature raise will first affect hydrogen bond that lead to denaturation before start breaking other bonds.

12. The calculation of temperature to dissociation also confirmed that the loosing of electrical conductivity is due to the full denaturation of λ-DNA and not by the dissociation of the thiol bond between the oligo and the electrodes or other bond that break the DNA into fragments.

We submit that the low impedance of λ-DNA molecular wires observed at moderate to high frequencies with the effect of temperature may have significant implications to the field of DNA-based bionanoelectronics.
REFERENCES


Aran, K., Sasso, L.A., Kamdar, N., Zahn, J.D. "Irreversible, direct bonding of nanoporous polymer membranes to PDMS or glass microdevices." *Lab Chip* 2010, 10, 548.


## APPENDIX A

### Table 9. Summary of Reported Results in Literature on DNA conductivity

<table>
<thead>
<tr>
<th>Researchers</th>
<th>DNA Specific Type &amp; Length</th>
<th>Electrode Type</th>
<th>Electrode/DN A contact</th>
<th>Substrate</th>
<th>Charge Transport</th>
<th>DNA as a Charge Carrier</th>
<th>Electrode Properties</th>
<th>Measurement Set-up</th>
<th>Measurement Condition</th>
<th>Resistance</th>
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</thead>
<tbody>
<tr>
<td>Dagano, Garcia et al. [22]</td>
<td>x-DNA</td>
<td>Pt (~20 nm gap)</td>
<td>Au with 40 nm gap</td>
<td>mica</td>
<td>Tunneling</td>
<td>DNA as charge carrier</td>
<td>Electrode trapping</td>
<td>STM tip</td>
<td>Dry</td>
<td>1.4 MΩ</td>
</tr>
<tr>
<td>Dr. Pablo Maintz, Menenno et al. [23]</td>
<td>x-DNA, 1.5 μm + 1 pm</td>
<td>Au with 40 nm gap</td>
<td>Au with 100 nm gap</td>
<td>mica</td>
<td>Tunneling</td>
<td>DNA as charge carrier</td>
<td>Electrode trapping</td>
<td>STM tip</td>
<td>Dry</td>
<td>1.1 MΩ</td>
</tr>
<tr>
<td>Nanarro, Marano, Menenno et al. [24]</td>
<td>x-DNA and poly(dC)</td>
<td>Pt (~20 nm gap)</td>
<td>Au with 40 nm gap</td>
<td>mica, glass, and silicon substrate</td>
<td>Tunneling</td>
<td>DNA as charge carrier</td>
<td>Electrode trapping</td>
<td>STM tip</td>
<td>Dry</td>
<td>1.1 MΩ</td>
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<tr>
<td>Poyhan et al. [25]</td>
<td>DNA covered with Au-DNA - SNWT</td>
<td>Au+ WIN &amp; Au-YNT</td>
<td>Au with 40 nm gap</td>
<td>mica</td>
<td>Tunneling</td>
<td>DNA as charge carrier</td>
<td>Electrode trapping</td>
<td>STM tip</td>
<td>Dry</td>
<td>1.1 MΩ</td>
</tr>
<tr>
<td>Elgala &amp; Poyhan et al. [26]</td>
<td>DNA(A) (poly(dA))</td>
<td>Au with 40 nm gap</td>
<td>Au with 100 nm gap</td>
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<td>DNA as charge carrier</td>
<td>Electrode trapping</td>
<td>STM tip</td>
<td>Dry</td>
<td>1.1 MΩ</td>
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<td>Car et al. [27]</td>
<td>DNA(DNA-DNA)</td>
<td>Au with 40 nm gap</td>
<td>Au with 100 nm gap</td>
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<td>Tunneling</td>
<td>DNA as charge carrier</td>
<td>Electrode trapping</td>
<td>STM tip</td>
<td>Dry</td>
<td>1.1 MΩ</td>
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<td>Katsiampis et al. [28]</td>
<td>DNA(A) (poly(dA))</td>
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<td>Au with 100 nm gap</td>
<td>mica</td>
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<td>DNA as charge carrier</td>
<td>Electrode trapping</td>
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<td>Dry</td>
<td>1.1 MΩ</td>
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<td>Braun et al. [29]</td>
<td>DNA(A) (poly(dA))</td>
<td>Au with 40 nm gap</td>
<td>Au with 100 nm gap</td>
<td>mica</td>
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<td>DNA as charge carrier</td>
<td>Electrode trapping</td>
<td>STM tip</td>
<td>Dry</td>
<td>1.1 MΩ</td>
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<tr>
<td>Fischer &amp; Schönhöfer [31]</td>
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<td>Au with 100 nm gap</td>
<td>mica</td>
<td>Tunneling</td>
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<td>1.1 MΩ</td>
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<tr>
<td>Mazzorano et al. [30]</td>
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<td>Electrode trapping</td>
<td>STM tip</td>
<td>Dry</td>
<td>1.1 MΩ</td>
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<tr>
<td>Gold &amp; Schönhöfer [31]</td>
<td>DNA(A) (poly(dA))</td>
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<td>Au with 100 nm gap</td>
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<td>DNA as charge carrier</td>
<td>Electrode trapping</td>
<td>STM tip</td>
<td>Dry</td>
<td>1.1 MΩ</td>
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<tr>
<td>Current Good et al. [32]</td>
<td>DNA(A) (poly(dA))</td>
<td>Au with 40 nm gap</td>
<td>Au with 100 nm gap</td>
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<td>Electrode trapping</td>
<td>STM tip</td>
<td>Dry</td>
<td>1.1 MΩ</td>
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</table>

Note: Data for DNA conductivity in literature is summarized in Table 9. The table includes researchers, DNA specific type and length, electrode type, electrode/DN A contact, substrate, charge transport, DNA as a charge carrier, electrode properties, measurement set-up, measurement condition, and resistance. The data shows variations in methodological approaches and results.
APPENDIX B

NEGATIVE LITHOGRAPHY PROCEDURE FOR MITHRAS ELECTRODES

Table 10. Negative Lithography with Metal Sputtering Protocol

<table>
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<tr>
<th>Step</th>
<th>Materials</th>
<th>Parameters</th>
<th>Procedural Description</th>
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</thead>
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<tr>
<td>1. Cleaning Si Substrate</td>
<td>Acetone, Isopropanol, DI water, air gun</td>
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<td>Rinse Si surface with acetone, then isopropanol, and then DI water. Use air gun to dry.</td>
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<tr>
<td>2. Dehydration bake</td>
<td>Hot Plate</td>
<td>2 min at 120°C</td>
<td>Place wafer on hot plate at 200°C for 15 min</td>
</tr>
<tr>
<td>3. Spin Coat</td>
<td>Spin Coater SU-8(10) or SU8(100)</td>
<td>45 sec @ 3000 rpm</td>
<td>Center wafer on chuck and apply 1mL per 1 in2 of wafer of SU-8. Spin coat at 3000 rpm for 45 sec.</td>
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<tr>
<td>4. Soft Bake</td>
<td>Hot plate</td>
<td>45-95°C over 50 min; 95°C for 5 min</td>
<td>Place wafer on hotplate at 45°C. Increase 5 degrees every 10 minutes. Hold at 95°C for 5 minutes.</td>
</tr>
<tr>
<td>5. UV exposure</td>
<td>UV Lamp, Patterned Mask</td>
<td>10mw/cm² for 30 sec</td>
<td>Place wafer on mask aligner and turn on suction to hold down wafer. Align mask on wafer and expose.</td>
</tr>
<tr>
<td>6. Post Bake</td>
<td>Hot plate</td>
<td>50-100°C over 30 minutes</td>
<td>Place wafer on hotplate at 50°C. Ramp up for 30 minutes to reach 100°C, and leave it for 5 minutes</td>
</tr>
<tr>
<td>7. Metal Sputtering</td>
<td>Metal Sputter Platinum target</td>
<td>6 nm Platinum, 6 nm gold</td>
<td>Place the wafer into the center of sputtering chamber. Run for 6nm Platinum. Change target with gold</td>
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</table>
Gold Target

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>8. Develop</td>
<td>Place wafer in developing solution for at least one minute. Agitate slightly. Remove from solution and rinse with Acetone. If white film shows up, place back in solution. Otherwise, wash with water and dry with air gun.</td>
</tr>
<tr>
<td>9. Image</td>
<td>Use 3D and 2D imaging to assess quality of features.</td>
</tr>
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</table>
# APPENDIX C

## DNA ATTACHMENT PROTOCOL

Table 11. DNA Attachment Method for Open Reservoir

<table>
<thead>
<tr>
<th>Process Type</th>
<th>Process Detail</th>
<th>Chemical &amp; Material</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annealing of Oligos</td>
<td>1. Serial dilution of both oligos from 500µM/µL to 1 µM/µL. It is done by twice of 10x dilution and one time of 5x dilution in a room temperature. 2. Mix same volume of both Oligo A and Oligo B in a 2 mL microtube. Normally we do 50 µL each. 3. Place tube in a water bath that is heated to around 105°C for 5 min. Assume that the temperature inside the tube will be around 95°C. 4. Remove the tube from the water bath and allow it to cool to room temperature (it takes around 30-45 minutes). 5. Store in a refrigerator at 4°C until use.</td>
<td>- Oligo A &amp; Oligo B (original concentration = 500 µM/µL)</td>
<td>Most of the time, we use stored annealed oligo</td>
</tr>
<tr>
<td>Reduction of Oligos</td>
<td>To use the free thiol (–SH), the disulfide linkage must be reduced with TCEP gel. 1. Take 40 µL of TCEP solution, vortex it and leave for 5 minutes for the slurry to condense and separate from the supernatant. Then remove the supernatant. Use composition of 1:1 for the TCEP vs Oligonucleotide for the reduction. 2. Take 40 µL of annealed oligo and mix it with the TCEP slurry. 3. Incubate at room temperature for 10 minutes for reaction and the solution</td>
<td>- Annealed Oligos - TCEP</td>
<td>Reduced oligo will have active of around 10 minutes. Oligo reduction is also done at room temperature</td>
</tr>
</tbody>
</table>
to separate from the slurry as we avoid centrifugation.

4. Take the supernatant as the reduced oligonucleotides

- Plasma etching of the Mithras chip will increase its wettability. Plasma etching is done at 75 Watt for 7 seconds.
- Drop 1 μL of reduced oligo on the center on the chip and leave it for 30 minutes.

**Oligo Attachment**

---

**λ-DNA end repair.**

Purify the DNA to be blunt-ended.

Combine and mix the following components in a microfuge tube (standard reaction).

We use standard 30 μl reactions that will end-repair up to 12.5 μg of DNA (DNA original concentration is 0.5 μg/μL)

The reaction can be scaled up or down as necessary.

1. Mix 25μL of linear, sheared and concentrated λ-DNA + 3μl of T4 Ligase buffer + 1μl of 10mM dNTPs + 0.5μl of T4 Polymerase + 0.5μL of T4 PNK added in 2 ml microfuge tube.
2. Incubate at room temperature for 45 minutes.
3. Stop the reaction by heating at 70°C for 10 minutes

The end-repaired DNA can be used for DNA ligation without purification. The
blunt-end ligation reaction can be performed for 45 minutes to 2 hours at room temperature.

4. Do series of serial dilution to have DNA in many different concentrations (500, 50, 25, 5, 2.5, 1, 0.5 and 0.25ug/μl).

5. Repaired DNA can be stored at 4°C until it use

DNA Ligase preparation

1. Mix 1μL of DNA Ligase with 4μL of DNA Ligase buffer. Composition of 1:4 can be scale up or down depends on how many chips we have

- T4 DNA Ligase
- T4 DNA Ligase Buffer

Ligation of λ-DNA + Oligos on the chip

Ligation of λ-DNA + oligos done by the adding all 3 together along with DNA ligase mix. Perform the blunt-end ligation reaction for 45 minutes to 2 hours at room temperature.

1. Add a drop of 1μL of Ligation mix on the Mithras chips that already have a drop of oligo from the earlier step.
2. Followed by adding a drop of 1μL of λ-DNA on the same chips.
3. Incubate for 45 minutes to 2 hour for the ligation to take place that means the DNA attached to the oligo and the bridge between two electrodes is formed. In our experiment, the ligation is done for 1 hour.

- Oligo already on the Mithras
- Blunt λ-DNA DNA Ligase mix

Run voltage with measuring resistant for about 3-5 seconds every 15 minutes during the 1 hour attachment time

After Attachment

1. After 1 hour of ligation, wash the attachment area with DI water to remove the excess of buffer. Deposit of dry salts can be observed during this period. The washing is done by
dropping 2μL of DI water using pipet to avoid strong force that can wash attached DNA away.

2. Use lint free wipes to absorb excess of solution and leave it to dry.
3. Do impedance measurement with our standard setting right after the washing and after every hour. The dry condition is indicated by stabilization of impedance measured. Initially the impedance measured will be in the range of $1.5 - 4\text{MOhm}$ as the DNA is covered with water molecules and the impedance will gradually increase every time since it will has less water contents. The dry condition is normally obtained after $>4$ hours and the impedance measured will be stable at $> 20\text{MOhm}$.
4. During the drying process, the chips are stored in a closed box at room temperature.
APPENDIX D

DNA ATTACHMENT PROTOCOLS
(MICROFLUIDICS-ASSISTED)

Table 12. DNA Attachment Method for Microfluidics-assisted Attachment

<table>
<thead>
<tr>
<th>Process Type</th>
<th>Process Detail</th>
<th>Chemical &amp; Material</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annealing of Oligos</td>
<td></td>
<td></td>
<td>Same as the open attachment</td>
</tr>
<tr>
<td>λ-DNA end repair</td>
<td></td>
<td></td>
<td>Same as the open attachment</td>
</tr>
<tr>
<td>DNA Ligase preparation</td>
<td>Mix 1μL of DNA Ligase with 4μL of DNA Ligase buffer and water. Composition of 1:4:15 can be scale up or down depends on how many chips we have</td>
<td>- T4 DNA Ligase</td>
<td>Should not be stored</td>
</tr>
<tr>
<td>Ligation of λ-DNA + Oligos in the tube</td>
<td>Ligation of λ-DNA + oligos done by the adding all 3 together along with DNA ligase mix. Perform the blunt-end ligation reaction for 45 minutes to 2 hours at room temperature.</td>
<td>- T4 DNA Ligase Buffer</td>
<td>Room Temperature</td>
</tr>
<tr>
<td></td>
<td>1. Add a drop of 10μL of Ligation mix into the tube that already have a 10μL of oligonucleotide solution from the earlier step.</td>
<td>- Oligo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. Followed by adding a drop of 10μL of the blunt end λ-DNA into the same tube.</td>
<td>- Blunt λ-DNA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Incubate for 45 minutes to 2 hour for the ligation to take place that means the DNA attached to the oligonucleotide, the ligation is done for 1 hour.</td>
<td>- DNA Ligase mix</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Followed by serial dilution of the</td>
<td></td>
<td></td>
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</tbody>
</table>
ligated DNA solution to meet the required concentration for experiment (from 100ng/μL to 1ng/μL)

Reduction of Oligos

To use the free thiol (–SH), the disulfide linkage must be reduced with TCEP gel.

1. Take 100 μL of TCEP solution, vortex it and leave for 5 minutes for the slurry to condense and separate from the supernatant. Then remove the supernatant.

Use composition of 1:1 for the TCEP vs Oligo for the reduction.

2. Take 100 μL of ligated DNA mixture and mix it with the TCEP slurry.

3. Incubate at room temperature for 10 minutes for reaction and the solution to separate from the slurry as we avoid centrifugation.

4. Take the supernatant as the reduced DNA solution with active thiol end

- Annealed Oligos
- Reduced Oligos
- TCEP

DNA Attachment

1. Input 100μL of the reduced DNA solution to the microfluidics chip that is connected to the peristaltic pump and let it circulate for about 10 minutes with changing direction every minute.

- Bali Mithras chip
- Reduced DNA solution

Reduced oligo will have active of around 10 minutes. Oligo reduction is also done at room temperature.

1. After 1 hour of circulation, unplug the tube while keep the pump on to rinse the solution.

2. Wash the channel with DI water to remove the DNA solution. The speed circulation shold remain the same or even slower.

3. Unplug the tube again to rinse the water then let the pump on to keep flowing air into the channel.

4. Do impedance measurement with our standard setting right after the washing and after every hour. The dry condition is indicated by stabilization of impedance measured. Initially the impedance measured will be in the range of 1.5 – 4MOhm as the DNA is covered with water molecules and the impedance will gradually increase every time since it will has less water contents. The dry condition is normally obtained after >4 hours and the impedance measured will be stable at > 20MOhm.
# APPENDIX E

## CHIPS INFORMATION

**Table 13. Chips Numbering and Experiment Information**

<table>
<thead>
<tr>
<th>Experiment #</th>
<th>Experiment Date</th>
<th>Chip #</th>
<th>DNA Dilutions Times (x)</th>
<th>DNA Concentration ng/ml</th>
<th>Attachment Evidence</th>
<th>Remarks</th>
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- Fluidics
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- First fluidic visual attachment
- Concentration variation & Temperature variation purpose
- Temperature variation purpose
- Concentration variation & Temperature variation purpose
- SEM, EDS
- Hyteresis & Renaturation
APPENDIX F
RENATURATION PROTOCOL

Tm Buffer is at pH 7.0 and contains:

a. 10 mM NaCl4
b. 10 mM Sodium Phosphate Buffer5
c. 0.1 mM EDTA6

Renaturation in PBS Solution
Once the conduction through all the devices was lost upon heat cycling, the chips were incubated again in Tm buffer solution. For this step, a vial containing the solution and chip was placed in a water bath and heated to 90 ºC. Once the temperature was stabilized, it was kept at 90 ºC for 10 minutes and cooled down slowly to room temperature. The chip was left immersed in the buffer overnight. The heating and cooling ramp in solution would provide the required cations for the re-hybridization of the ss-DNA [162]. After this second incubation, the chips were dried in N2 ambient and again I-V measurements were done. The conduction was regained in a few devices. The yield at this step was very low and about 10% devices showed this behavior, but this confirmed that the conduction was disrupted due to the denaturing of the DNA and it had been re-gained.
ABSTRACT OF THE THESIS

DNA Molecular Wire-Based Nanoelectronics: New Insight and High Frequency AC Electrical Characterization
by
Denni Ari Wibowo
Master of Science in Bioengineering
San Diego State University, 2014

While recent research in electron-transport mechanism on a double strands DNA seems to converge into a consensus, experiments in direct electrical measurements on a long DNA molecules still lead to a conflicting result. This research investigates experimentally the attachment of DNA molecular wire to high aspect ratio three-dimensional (3D) metal electrode and the effect of temperature to its AC electrical conductivity. The 3-D microelectrode was built on a silicone oxide substrate using patterned thick layers of negative tone photoresist covered by sputtered gold on the top surface. Attachment of λ-DNA to the microelectrode was demonstrated using oligonucleotide-DNA phosphate backbone ligation and thiol-gold covalent bonding. Electrical characterizations based on I-V and AC impedance analysis of several repeatable data points of attachment with varying λ-DNA concentration (500 ng/µL to 0.0625 ng/µL) showed measurable and significant conductivity of λ-DNA molecular wires. Further study was carried out by measuring I-V and impedance while ramping up the temperature to reach complete denaturation (~110°C) resulting in no current transduction. Subsequent re-annealing of the DNA through incubation in TM buffer at annealing temperature (~90°C) resulted in recovery of electrical conduction, providing a strong proof that DNA molecular wire is the one generate the electrical conductivity. λ-DNA molecular wires reported to have differing impedance response at two temperature regions: impedance increases (conductivity decrease) between 4°C – 40°C, and then decreases from 40°C until DNA completely denatured (~110°C). The increase conductivity after 40°C is an experimental support the long distance electron transport mechanism referred as “thermal hopping” mechanism. We believe that this research represents a significant departure from previous studies and makes unique contributions through (i) modification of DNA attachment methods has increase the success rate from less than 10% to be more than 75% (ii) more accurate direct conductivity measurement of DNA molecular wires facilitated by suspension of the DNA away from the substrate, and (iii) AC impedance measurement of DNA
molecular wires with the effect of temperature suggests an experimental evidence of temperature gating mechanism in charge transport through DNA wire that will be very important for further studies.