DNA MANIPULATION AND CHARACTERIZATION FOR NANOSCALE ELECTRONICS

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Reported measurements of DNA conductivity thus far have investigated this biomolecule in its native, undamaged double helical form. This work presents unique comparative measurements on differently modified DNA strands, to determine the relationship that exists between the molecule’s structure and its electronic properties.

Disulfide-labeled λ-DNA molecules were aligned between Au electrodes via dielectrophoresis and current-voltage (I-V) characteristics were measured. Comparisons were made between the characteristics of undamaged, double helical λ-DNA and λ-DNA with varying quantities of nicks introduced into its phosphate backbone. The undamaged DNA gave a close-to-linear I-V characteristic with an estimated dc conductivity of $1 \times 10^{-3} \text{ S cm}^{-1}$. In contrast, DNA with two nicks gave a pronouncedly non-linear curve with a conductivity gap of $\sim 3 \text{ eV}$. Each nick was present in only one strand of the helix and since flow of current was measured, this indicated the involvement of the undamaged complementary strand in charge transport through the molecule. Furthermore, the size of the gap increased with the number of nicks introduced. The conductivity gap, resulting from the presence of nicks, could be reduced by utilizing T4 DNA ligase to repair the backbone even after the DNA was aligned on the device. These results indicate that enzymatic manipulation of the molecule’s structure can be utilized for applications in nanoscale electronics.
In addition, the $I-V$ characteristics of disulfide-labeled single stranded λ-DNA molecules (ssλ-DNA) were measured. The ssλ-DNA was formed using two different methods for comparison purposes: a thermal/chemical denaturation and an enzymatic digestion utilizing λ-exonuclease. Resulting $I-V$ curves, in both instances, were close-to-linear when measured at room temperature. However, the ssλ-DNA samples consistently gave conductivity values approximately two orders of magnitude smaller in amplitude than the values obtained for undamaged, disulfide-labeled double stranded λ-DNA.

These results suggest an integral relationship between the native structure of DNA with its stacked base pairs and the molecule’s ability to support charge transport. Hence, methods of modifying base pairs, and ensuing $\pi$-orbital overlap were explored. These included the synthesis of perfectly repetitive DNA sequences and the incorporation of transition metal cations between base pairs.

Approved:

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<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AFM</td>
<td>atomic force microscopy</td>
</tr>
<tr>
<td>B-DNA</td>
<td>B-conformation DNA</td>
</tr>
<tr>
<td>bp</td>
<td>base pair</td>
</tr>
<tr>
<td>CE</td>
<td>capillary electrophoresis</td>
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<tr>
<td>DLA</td>
<td>diffusion limited aggregation</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>DNAse I</td>
<td>Deoxyribonuclease I</td>
</tr>
<tr>
<td>dsλ-DNA</td>
<td>double stranded λ-phage DNA</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiotreitol</td>
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<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EOF</td>
<td>electroosmotic flow</td>
</tr>
<tr>
<td>HEC</td>
<td>hydroxyethylcellulose</td>
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<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>IDT</td>
<td>Integrated DNA Technologies</td>
</tr>
<tr>
<td>I-V</td>
<td>current-voltage</td>
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<tr>
<td>λ-DNA</td>
<td>λ-phage DNA</td>
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<tr>
<td>LIF</td>
<td>laser induced fluorescence</td>
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<tr>
<td>M-DNA</td>
<td>metallated DNA</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PDMA</td>
<td>poly(dimethylacrylamide)</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>POP-4</td>
<td>Performance Optimized Polymer 4</td>
</tr>
<tr>
<td>PVP</td>
<td>poly(vinylpyrrolidone)</td>
</tr>
<tr>
<td>RFU</td>
<td>relative fluorescence units</td>
</tr>
<tr>
<td>RH</td>
<td>relative humidity</td>
</tr>
<tr>
<td>SAM</td>
<td>S-adenosylmethionine</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
</tr>
<tr>
<td>ssλ-DNA</td>
<td>single stranded λ-phage DNA</td>
</tr>
<tr>
<td>STR</td>
<td>short tandem repeat</td>
</tr>
<tr>
<td>TAPS</td>
<td>N-tris(hydroxymethyl)-methyl-3-aminopropanesulfonic acid</td>
</tr>
<tr>
<td>TBE</td>
<td>tris-borate-EDTA</td>
</tr>
<tr>
<td>TEAA</td>
<td>triethylammonium acetate</td>
</tr>
<tr>
<td>Tris</td>
<td>tris(hydroxymethyl)aminomethane</td>
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</table>
Chapter 1. General Introduction

1.1. Molecular Electronics

The microelectronics industry is continually pushing for the development of smaller components and circuitry to increase chip functionality and reduce cost.\(^1\) As a result, it is anticipated that by 2015 the size of the individual devices on these chips will be ~25 nm.\(^2\) However, it has been widely projected that at these dimensions, traditional “top-down” lithographic methods for manufacturing microelectronics will have reached their resolution limits.\(^3\) One potential solution to this problem is to replace conventional microelectronic circuitry with nanoscale devices developed using “bottom-up” or self-assembly processes.\(^4\) For this reason, there has been much interest in molecular-scale electronics, i.e. electronic devices that use individual molecules as wires, switches, rectifiers, and memories.\(^5\) In this context, the use of deoxyribonucleic acid (DNA) molecules to provide inter-element electrical connections and to interface these nanoscale circuits to macroscopic electrodes, is very promising.\(^6\)

1.2. Brief Background on DNA

The idea that DNA may be able to effectively transport charge was proposed more than 40 years ago with the discovery of the molecule’s double helix conformation.\(^7\) A single DNA strand consists of a repeating phosphate-deoxyribose sugar backbone, linking together combinations of four different aromatic nitrogenous bases: adenine (A), thymine (T), guanine (G), and cytosine (C), Figure 1-1. Adenine and guanine are double ring structures called purines, while thymine and cytosine are single ring structures called pyrimidines. The phosphate group attached to the number-5 carbon atom (5´ carbon) of
**Figure 1-1.** Schematic representation of the DNA sugar-phosphate backbone and the four bases. Adapted from Reference 6.
the deoxyribose of one deoxyribonucleotide is connected to the number-3 carbon (3\textsuperscript{\prime} carbon) of the adjacent deoxyribonucleotide. Thus, each DNA strand has directionality. The end of the DNA strand that terminates with the 5\textsuperscript{\prime} carbon atom is called the 5\textsuperscript{\prime} end and the end with the 3\textsuperscript{\prime} carbon atom is called the 3\textsuperscript{\prime} end. The elegant structure of the double helix is formed when two single strands are held together by hydrogen bonding between Watson-Crick base pairs: A-T and G-C, as depicted in Figure 1-2 (a). For this hydrogen bonding to occur, the strands must be antiparallel to one another so that one strand goes from 5\textsuperscript{\prime} to 3\textsuperscript{\prime} and the other from 3\textsuperscript{\prime} to 5\textsuperscript{\prime}. The base pairs, which are flat, planar structures, stack on top of one another at the core of the double helix as shown in Figure 1-2 (b).\textsuperscript{8} This stacking confers additional stability to the molecule by preventing water molecules from entering the spaces between the base pairs.\textsuperscript{6} The exact helical structure that the DNA molecule adopts can vary. At physiological conditions most of the DNA in bacterial or eukaryotic cells exists in the B-DNA form, a right-handed helix with 10 base pairs per turn and a separation distance of 3.4 Å between pairs.\textsuperscript{9} The diameter of the double helix in this form of DNA is 20 Å.

1.3. Initial Charge Transfer Studies

The unique structure of DNA allows it to fulfill vital biological roles including its own replication and the storage of genetic information. In 1962, Eley and Spivey were the first to propose that the $\pi-\pi$ orbital overlap that exists between its stacked base pairs could also allow for efficient charge migration.\textsuperscript{7} This geometry strongly resembles conductive one-dimensional aromatic crystals such as the Bechgaard salt, (TMTSF)$_2$PF$_6$.
Figure 1-2. (a) Schematic representation of DNA base pairing. (b) Schematic representation of DNA double helical structure. Adapted from Reference 9.
where TMTSF is tetramethyltetraselenafulvalene (Figure 1-3).\textsuperscript{6,10,11} The first experiments used to probe the electronic properties of DNA involved the measurement of current flow in DNA fibers.\textsuperscript{7,12} These early studies suggested that DNA behaved as a semiconductor. However, it was not until Barton’s group published papers in the early 1990’s that widespread interest in the ability of this biomolecule to support charge transfer and transport, really arose.\textsuperscript{13-15}

Barton’s group initially employed photochemical methods to determine the molecule’s electrical properties while in solution.\textsuperscript{13,15,16} Their oligonucleotides contained an electron donor and an acceptor and electron transfer was measured through fluorescence quenching as a function of distance. In one of the pioneering experiments conducted by Barton and her colleagues, a ruthenium intercalator was tethered near the 5´ end of one DNA strand and a rhodium intercalator was bound near the 5´ terminus of the complementary strand.\textsuperscript{15} The two strands were then annealed and the ruthenium complex was photoexcited. Within a tenth of a nanosecond, the ruthenium luminescence was completely quenched by the rhodium intercalator that was located 4 nm down the duplex. From this and additional experiments they completed, it appeared that DNA could serve as a molecular bridge for electron transfer between metallointercalators, with rapid quenching over long distances. However, this result was in conflict with standard electron transfer theory.\textsuperscript{11} In 1985, Marcus developed a theory which predicted an exponential rate decrease of the electron transfer step with increasing distance, i.e. $k \propto e^{-\beta r}$, where $r$ is the separation between the donor and the acceptor and $\beta$ represents the
Figure 1-3. Schematic of the Bechgaard salt (TMTSF)$_2$PF$_6$, a conductive one-dimensional aromatic crystal. The planar organic molecules are stacked along the a-axis; in the c-direction they are separated by the PF$_6$-anions. Adapted from Reference 6.
efficiency of the transfer.\textsuperscript{17,18} This exponential decay in the transfer rate has been observed in many proteins and straight chain aliphatic groups, giving $\beta$ values from $\sim$0.85 to 1.2 Å$^{-1}$.\textsuperscript{8} Barton’s data yielded $\beta$ values of less than 0.2 Å$^{-1}$, an incredibly small number that implied distinct wire-like behavior.\textsuperscript{15} Furthermore, not only did this work disagree with theory, other experimental studies showed that photoinduced electron transfer in this biomolecule was strongly distance dependent.\textsuperscript{19,20} For example, Lewis et al. examined synthetic DNA hairpins that were linked at one end by stilbene and measured photoinduced electron transfer between the stilbene acceptor and a guanine donor.\textsuperscript{20} His group instead found $\beta$ values of 0.64 ± 0.1 Å$^{-1}$.

It now appears that these contradicting results were due to the high sensitivity of the electron transfer reaction to the coupling between the donor and acceptor probes and the DNA strand.\textsuperscript{13,16} Donors and acceptors that were well stacked or intercalated into the DNA helix (as in Barton’s experiments) gave results demonstrating fast electron transfer rates with minimal distance dependence.\textsuperscript{21} On the other hand, when the probes were not as well incorporated into the DNA helix and instead connected through $\sigma$-linkages or limited base stacking, the reactions proceeded more slowly, with a strong distance dependence. This theory was supported by further experiments that Barton and colleagues completed which showed that when base mismatches or other stacking perturbations were introduced into the helix, long-range electron transfer was prevented.\textsuperscript{16,22,23} This additional work also gave strong evidence that electrons were indeed traveling through the $\pi$-$\pi$ stack of the base pairs.
Biochemical studies have also been useful in establishing that DNA charge transfer can proceed over long distances (>50 Å). In these studies a photo-oxidant like anthraquinone was attached to a DNA strand at a given position separated from a guanine doublet or triplet. Guanine has the lowest oxidation potential of the four bases and therefore, it is the target of oxidative damage. Furthermore, GG or GGG sequences have even lower oxidation potentials than single G’s. In this situation one electron can be removed from the DNA, i.e. the guanine base, and transferred to the photoexcited oxidant. This process is described as “hole transfer.” By analyzing the yield of strand breaks resulting from this oxidative damage using gel electrophoresis, researchers could establish that this mechanism of charge transfer was occurring. In these assays, the effects of intervening bulges, DNA-binding proteins, and variations in sequence and length were also examined. Because of the results of both biochemical and photochemical studies, it is generally accepted that hole and electron transfer can occur through DNA and research is now focused on its biological relevance.

1.4. Reasons for Studying DNA Charge Transfer

1.4.1. Biological Importance

For biologists, DNA-mediated electron or hole transfer is of interest because of its relationship to DNA damage and repair. As already mentioned, researchers have established that hole transfer through DNA can promote oxidative damage to guanine bases from distant sites. However, whether long-range oxidative damage is an issue of physiological importance within the cell has not yet been determined. It is known that when a hole is injected into DNA due to interaction of the molecule with ultraviolet (UV)
radiation or exposure to oxidants, this positive charge can be transferred from a single G\(^{+*}\) to GG or GGG sequences, Figure 1-4.\(^{18}\) During this process, the normal guanine is converted to 8-oxoguanine or other products.\(^{34}\) This results in a mutation that has been shown to lead to errors in the DNA replication process. Specifically, adenine instead of cytosine can be incorporated into the complementary strand and a transversion from a G:C base pair to a T:A base pair can occur.\(^{34}\) Several important codons of p53 tumor suppressor genes along with proto-oncogenes contain G clusters in their sequences and therefore, these mutations can potentially increase carcinogenesis.

Studies have also shown that DNA electron transfer can be utilized to repair UV-induced damage.\(^{35}\) For example, Carell and colleagues synthesized DNA strands that contained a cyclobutane thymine dimer lesion and a flavin derivative incorporated synthetically into the oligonucleotide as an artificial nucleobase.\(^{35,36}\) They found that when the flavin was in its reduced, photoexcited state, it was capable of causing the cycloreversion of the dimer lesion via electron transfer.

Similarly, Dandliker et al. observed that the cyclobutane thymine dimer could also be repaired over a distance of at least 26 Å using an intercalating rhodium complex to serve as a strong photooxidant.\(^{37}\) Therefore, repair of these lesions appears to proceed through both radical cation and radical anion intermediates. These results may have important implications for skin cancer patients.\(^{35}\) Certain compounds capable of repairing mutagenic dimer lesions could be bound to DNA and via charge transfer, rescue cells from UV-induced cell death.
Figure 1-4. Oxidation of a single G and subsequent hole transfer to a mutational target, a G triplet. Adapted from Reference 18.
Several hypotheses have been suggested to explain the natural function of DNA’s *in vivo* electronic properties. Barton and colleagues have explored the idea that charge migration might be involved in DNA damage recognition by an organism’s repair system. They propose that redox-active signal and receiver enzymes could work together as a pair to scan the genome for damage, utilizing the π-stack to mediate long-range redox interactions. Undamaged DNA would facilitate long range oxidation and therefore have the ability to reduce the receiver protein. Like other transcription factors, the oxidation of the signal enzyme could cause this protein to dissociate from the DNA substrate and relocate to another region of DNA (reduced signal enzymes can reattach downstream). However, if a damaged DNA region was present between the pair, the signal enzyme would proceed down the helix until it reached the site of local disruption. Hence, the absence of DNA-mediated electron transfer would prevent enzyme dissociation and the sampling of new sites, forcing the enzyme to instead locate and “mark” the sites in need of repair.

Another interesting hypothesis has been proposed by Heller. As previously mentioned injected holes have been shown to oxidize remote G sites, particularly those containing multiple G’s. Heller suspects that some species may have evolved chromosomal domains containing essential genes that are cathodically protected against oxidative damage incurred by substances like H₂O₂ and NO. This protection arises from G-rich DNA sequences at the termini of chromosomes, which are sacrificially oxidized when an oxidizing agent attacks the genome.
1.4.2. Nanoelectronics

While Barton and other biochemists designed experiments to study charge transfer through the DNA helix in solution, their results triggered interest from other scientific disciplines with regard to the possibility of using DNA-based electronic devices in a dry environment. It is logical to speculate that if charge transfer (a one-step tunneling process) can occur within DNA, transport (multiple transfer steps) is also a possibility, allowing DNA to be used as a so-called “molecular wire.” Other types of molecules that have been suggested as possible candidates for use as molecular wires include conjugated hydrocarbons, carbon nanotubes, and porphyrin oligomers. However, DNA offers some attractive advantages when compared to these other promising molecules. The very specific binding that occurs between complementary single stranded DNA, can be utilized for self-assembly purposes and therefore, many of the difficulties in positioning molecular electronics at the nanometer scale can be bypassed. For example, Seeman and his colleagues have utilized DNA’s self-assembly capabilities to create complex nanostructures including intertwined loops, knots, and three-dimensional cubes. The precision with which these structures can be fabricated is still far in advance of any direct manufacturing process. DNA is also advantageous as a nanostructural polymer because of its relatively high mechanical rigidity and physicochemical stability. Moreover, this biomolecule has an inherent programmability through variation of its nucleobase sequence. Finally, highly specific enzymes such as ligases, polymerases, and endonucleases can be utilized for very precise manipulation of the DNA strands before or after integration into electronic circuits.
1.5. Direct Measurements of Electrical Transport in DNA

Although DNA’s potential in molecular electronics has been recognized, its ability to serve as a pathway for charge transport remains controversial. Direct electrical transport measurements commenced in 1998 and since that time there have been many experiments completed, yielding a wide range of results. Researchers have found that DNA acts as a good conductor,\textsuperscript{41,43,46,47} semiconductor,\textsuperscript{42,48} insulator,\textsuperscript{40,49-51} or even a proximity-induced superconductor.\textsuperscript{52} The conflicting experimental results are summarized in Table 1-1 and can likely be attributed to various conditions, which are not kept constant among the different experiments performed. These critical factors include the surface of the substrate, contacts between the electrodes and DNA molecules, DNA sequence and strand length, counterions along the phosphate backbone, level of DNA hydration, and DNA secondary structure (bends, nicks, stacking distance between base pairs, and width of the DNA molecule).

1.6. Mechanisms of DNA Charge Transport

Not only have researchers been unable to come to a unanimous conclusion regarding DNA conductivity but also the mechanism of this long distance charge transport still remains the subject of active experimental and theoretical research.\textsuperscript{53-60} It is unclear whether the current that arises in response to the electric field applied is due to the overlapping electron orbitals of the stacked DNA base pairs, the positively charged, hydrated counterions along its phosphate backbone, or some combination of these mechanisms. Two mechanisms are generally accepted as the dominant means by which DNA could mediate charge transport along its stacked base pairs.\textsuperscript{1,10,42,61} Depending on
Table 1-1. Summary of results from direct measurements of DNA resistance.

<table>
<thead>
<tr>
<th>Class</th>
<th>Measurement method</th>
<th>Research group</th>
<th>DNA sample</th>
<th>Result(s)</th>
<th>Resistance calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductor</td>
<td>Low-energy electron point source microscope and tungsten manipulation tip, bundles free-hanging on Au-coated sample holder</td>
<td>Fink and Schönenberger (41)*</td>
<td>Bundles of λ-DNA segments DNA length= 600-900 nm</td>
<td>Linear at RTb</td>
<td>3 × 10⁶ Ω</td>
</tr>
<tr>
<td></td>
<td>DNA on mica substrate, one Au electrode deposited on top and used along with a conducting cantilever tip</td>
<td>Cai et al. (46)</td>
<td>Self-assembled networks of poly(A)/poly(T) and poly(G)/poly(C), DNA length= 100 nm</td>
<td>Linear and p-type rectifying characteristics (G/C only) at RT</td>
<td>5-20 × 10⁹ Ω</td>
</tr>
<tr>
<td></td>
<td>Contactless measurement of resonant cavity losses at microwave frequencies</td>
<td>Tran et al. (43)</td>
<td>λ-DNA, DNA length= 16 μm</td>
<td>Strongly temperature dependent transport, indicating a hopping mechanism</td>
<td>10¹³ Ω</td>
</tr>
<tr>
<td></td>
<td>DNA electrostatically trapped between Au electrodes on SiO₂</td>
<td>Yoo et al. (47)</td>
<td>Supercoiled poly(A)/poly(T) and poly(G)/poly(C), DNA length= 0.5-2.9 μm (folded to &lt;100 nm), electrode gap= 20 nm</td>
<td>Linear at RT, insulating at low temperature</td>
<td>1-100 × 10⁶ Ω</td>
</tr>
<tr>
<td>Semiconductor</td>
<td>DNA electrostatically trapped between Pt electrodes, free hanging</td>
<td>Porath et al. (42)</td>
<td>Single molecule, poly(G)/poly(C), DNA length= 10.4 nm, electrode gap= 8 nm</td>
<td>Large band-gap semiconductor at RT and in vacuum at cryogenic temperature</td>
<td>3 × 10⁹ Ω</td>
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<td>DNA connected between Au electrodes using &quot;gluing technique&quot; and flow alignment, bundles free-hanging</td>
<td>Rakitin et al. (48)</td>
<td>Bundles of λ-DNA, DNA length= 16 μm, electrode gap= 10 μm</td>
<td>Narrow band-gap semiconductor at RT</td>
<td>3 × 10⁸ Ω</td>
</tr>
<tr>
<td>Insulator</td>
<td>Connected between Au electrodes using &quot;gluing technique&quot; and flow alignment, bundles free-hanging</td>
<td>Braun et al. (49)</td>
<td>Single λ-DNA molecule, DNA length= 16 μm, electrode gap= 12 or 16 μm</td>
<td>Insulating at RT</td>
<td>&gt;10¹³ Ω</td>
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*Reference number

bNotation: RT= room temperature
<table>
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<tr>
<th>Class</th>
<th>Measurement method</th>
<th>Research group</th>
<th>DNA sample</th>
<th>Result</th>
<th>Resistance calculated</th>
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<tr>
<td>Insulator</td>
<td>DNA on mica substrate, Au electrodes deposited on top (for short λ, conductive</td>
<td>dePablo et al. (40)</td>
<td>Single λ-DNA molecule, DNA length= 1.8 or 16 µm, electrode gap= 4 µm</td>
<td>Insulating at</td>
<td>≥10^{12} Ω (short λ)</td>
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<td></td>
<td>cantilever tip used as 2nd electrode)</td>
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<td>(for short λ ≥ 70 nm)</td>
<td>RT</td>
<td>≥10^{16} Ω (long λ)</td>
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<td>Unlabeled DNA on mica substrate and thiol-labeled DNA on SiO₂ substrate,</td>
<td>Storm et al. (50)</td>
<td>More than 1 molecule: 1) λ-DNA labeled with thiol groups, DNA length= 300</td>
<td>Insulating at</td>
<td>&gt;10^{12} Ω</td>
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<td></td>
<td>Au or Pt electrodes</td>
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<td>nm and 1.5 µm, electrode gap= 40-500 nm and 2) poly(G)/poly(C), DNA</td>
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<td></td>
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<td>length= 2 µm, electrode gap= 100-200 nm</td>
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<td>Thiol-labeled DNA on quartz substrate, flow alignment, Au electrodes</td>
<td>Zhang et al. (51)</td>
<td>Approximately 1000 λ-DNA molecules, DNA length= 16 µm, electrode gap= 4 or</td>
<td>Insulating at</td>
<td>&gt;10^{16} Ω</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>8 µm</td>
<td>RT in vacuum (&lt;10^{-7} Torr)</td>
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<td></td>
<td>Superconductor</td>
<td>Kasumov et al. (52)</td>
<td>A few molecules of λ-DNA DNA length= 16 µm</td>
<td>Proximity-induced</td>
<td>&lt;10^{6} Ω</td>
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<td>DNA on mica substrate, flow alignment, connected by 2 superconducting electrodes</td>
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</table>

*aReference number

bNotation: RT= room temperature
the coupling strength between different base pairs, it is proposed that transport could take place through band-like conduction or multistep hopping.

In the case of duplex oligomers containing identical base pairs, i.e. poly(G)/poly(C), the π-electron states can overlap strongly, yielding an ideal periodic potential. Therefore, depending on the coupling strength between the adjacent potential wells (base pairs), band-like transport can take place. This process is said to be “coherent” because the charge does not exchange energy with the molecule during transport and the electronic states are delocalized over the entire length of the molecule, Figure 1-5 (a). This is similar to what can occur in doped semiconductors when the concentration of the impurity is large enough that the impurity states overlap one another, forming an “impurity band.” Barton and colleagues have demonstrated that under certain conditions charge transport can occur with little distance dependence, indicative of delocalized energy states and the band-like conduction mechanism.

In contrast, if the double stranded DNA instead consists of a completely random sequence, no band can exist because of the lack of a periodic potential. In this case, the migration of charge can instead occur by a multistep hopping mechanism. This process is said to be “incoherent” because the charge is localized on the molecule and can exchange energy with it. In the mechanism of DNA-mediated hole hopping, the guanine radical cation (G+) is proposed to serve as the intermediate hole carrier (since it has the lowest ionization potential of the four bases). Giese and Spichy have suggested that adenines can be utilized as the hole carriers if guanines are absent from the base sequence. After a positive charge is introduced into a DNA strand containing guanine
Figure 1-5. (a) Schematic illustrating band-like conduction. In this model, the base pair coupling is large due to the periodic DNA sequence. Therefore, the π-electron states overlap strongly, leading to the delocalization of the states over the molecule and the formation of a molecular band. (b) Schematic illustrating multistep hopping. As shown in the relative energy diagram, a positively charged hole is more stable (has a lower energy) on a G-C base pair than on an A-T base pair. Therefore, a hole will localize on a G-C base pair and since A-T base pairs have a higher energy, they act as barriers. The hole moves from one G-C pair to the next by incoherent hopping.
bases, it is thought to hop from G to G as shown in Figure 1-5 (b). Meggers et al. have established experimental support for this mechanism by analyzing the distance dependence of oxidative base damage after DNA was exposed to ionizing radiation. They found that a very steep decay existed for the yield of DNA damage when sequences did not contain guanine while G-rich DNA gave a very shallow decay.

Recently, γ-pulse radiolysis and photochemical studies have indicated that a hopping mechanism may also occur for electron transport within DNA. For example, Raytchev et al. found that efficient electron injection into oligonucleotides using pyrene as the electron donor only occurred when a thymine base was located near the donor. This result is consistent with an electron hopping mechanism because thymine is the most easily reduced base and therefore, should serve as the intermediate electron carrier.

In addition to these two mechanisms, Schuster and colleagues have proposed a modified multistep hopping method that takes into account conformational dynamics within the base stack. This “phonon-assisted polaron hopping mechanism” involves the formation of a transient polaron when a hole or electron is injected. A DNA polaron is a radical cation or anion that is delocalized over 5 to 7 base pairs. In this region the DNA structure is distorted and it is believed that there is a decrease in the distance between bases. Therefore, more overlap between the base molecular orbitals results and a minimization of cation or anion energy occurs. Thermal activation (phonons) then cause the polaron to undergo a hopping mechanism where base pairs with similar energy will leave or join, causing the polaron to travel through the DNA.
1.7. Project Objectives

The focus of this DNA research is three-fold: 1) to develop a procedure for the reproducible measurement of DNA electrical conductivity, 2) to investigate the method of charge transport in this biomolecule, and 3) to use this information to optimize DNA conductivity, therefore increasing the potential for this molecule to be used in nanoelectronics.

To address the first objective, new techniques were developed to align and attach DNA between electrodes reproducibly. In order to accomplish this, a method was established for labeling \( \lambda \)-phage DNA (\( \lambda \)-DNA) with disulfide end groups to improve the molecule’s electrical contacts to the gold electrodes used in \( I-V \) measurements. This procedure was adapted from Braun et al.\(^49\). Furthermore, a rigorous electrode cleaning technique was developed to maximize the formation of chemisorbed disulfide-Au bonds and thus, DNA attachment. Finally, DNA alignment between the electrodes was optimized by manipulation with ac electric fields.

Most of the DNA conductivity measurements completed thus far have investigated this biomolecule in its native, undamaged double helical form. However, in order to gain a better understanding of the mechanism of charge transport, it is necessary to directly explore the relationship between DNA structure and the molecule’s electronic properties. Therefore to address the second objective, comparative measurements were made on different forms of DNA, to determine how manipulating the structure of this molecule affected its measured current-voltage characteristics.\(^{71,72}\) Specifically, various structural forms of disulfide-labeled \( \lambda \)-DNA have been measured. Studies of nicked
versus repaired DNA along with a comparison of two different disulfide end group configurations, offer insight into the mechanism of charge transport in DNA. In addition, measurements and comparisons of single stranded $\lambda$-DNA (ss$\lambda$-DNA) versus double stranded $\lambda$-DNA (ds$\lambda$-DNA) have provided an even better understanding of the relationship between DNA structure and charge transport. The low conductivity values obtained for ss$\lambda$-DNA samples when compared to ds$\lambda$-DNA, suggest an integral relationship between the native double helical structure of DNA with its stacked base pairs and overlapping electron orbitals, and the molecule’s ability to support charge transport.

Since this research has demonstrated that the dominant component influencing charge transport is the stacked base pairs, DNA sequences have been manipulated to address the third area of this research and improve the conductivity of the molecule. Highly repetitive sequences of thiol-labeled DNA have been synthesized, including poly(A/T), poly(G/C), and poly(AT/TA), with ligated lengths compatible to electrode gap sizes. The effect of intercalated divalent metal cations on DNA conductivity has also been examined. Under specific conditions, certain divalent cations can replace the imino protons of the base pairs and covalently bind to the DNA bases, resulting in a metal ion-DNA complex termed M-DNA. It is speculated that by utilizing this method of modification, the electronic properties of these molecules can be enhanced. Capillary electrophoresis and atomic force microscopy have been used to characterize the structural effects of this metal binding, the stability of M-DNA once formed, and the relationship between DNA sequence and M-DNA formation. This information is important both for
successful $I-V$ characterization of M-DNA and for its potential use as an element in nanoelectronics.
Chapter 2. Experimental Procedures

Initially, this research focused on the synthesis and conductivity characterization of the doubly functionalized DNA fragment, D1S80. However, a visualization method could not be used to confirm the presence of these molecules and/or help in optimizing their alignment due to their short fragment length. The D1S80 length necessitated very narrow electrode gaps (120-180 nm) and therefore, for ease of visualization, research shifted to the measurement of a much longer fragment, $\lambda$-DNA. $\lambda$-DNA molecules could be aligned and attached across micrometer size electrode gaps, which allowed for their detection using confocal microscopy and a fluorescent intercalating dye.

2.1. D1S80 Synthesis and Characterization

The well-characterized DNA fragment called D1S80 is a repetitive marker that can be used in forensic identification due to its polymorphic nature in the human genome. D1S80 consists of 16 base pair (bp) repeats, with a total length varying between 369 and 801 bp. These alternative forms of the same DNA region are called alleles. Taking into account a distance of approximately 3.4 Å between each base in DNA, the length of the different allele possibilities range from about 125 to 272 nm.

2.1.1. FTA® Extraction

To prepare labeled DNA samples, template DNA was extracted from bloodstains on FTA® cards. FTA® cards are a commercially available product that consist of filter paper loaded with a proprietary mix of reagents that cause cells to lyse, prevent the growth of bacteria, and protect the DNA in the sample. After biological specimens were applied to the FTA® paper and air-dried, a small disc of the paper was removed.
This disc was then washed to remove any non-DNA material while the DNA remained entangled within the paper. Subsequent amplification was performed on the DNA while it was still attached to the paper.

2.1.2. Reduction of Disulfide-Labeled Primers

The D1S80 region of DNA was amplified by the polymerase chain reaction (PCR) using either unlabeled primers, thiol-labeled primers, or a thiol-labeled forward primer and an amine-modified reverse primer. Thiol-labeled primers were delivered from Integrated DNA Technologies (IDT) in the disulfide form and the various functional groups mentioned above were connected to the 5′ termini of the oligonucleotides via a six carbon linker (C6). A relatively simple cleavage protocol could be used to form the reduced thiol (-SH) labeled primer. This procedure involved reconstituting the oligonucleotide in 0.05 M dithiotreitol (DTT). Prior to use of the thiol-modified oligonucleotide, i.e. after PCR but before its conductivity measurement, ethyl acetate could be used to remove DTT from the sample. After initial attempts to measure thiol-labeled D1S80 samples failed, the extraction step was eliminated from all further sample preparations. Instead, DTT was left in the solution to minimize the formation of disulfide bonds between the thiol labels.

2.1.3. PCR Amplification

The Promega PCR Master Mix containing Taq DNA polymerase, dideoxy nucleotide triphosphates (dNTPs), and MgCl₂ in a proprietary reaction buffer was utilized for amplification. An annealing temperature of 65 °C was chosen on a GeneAmp® PCR System 9700 with a standard cycling program. This value corresponded to the
recommended annealing temperature of 5 °C below the calculated melting temperature of the primers.

2.1.4. CE Characterization

Amplified allele lengths were characterized using an ABI 310 Genetic Analyzer to perform capillary electrophoresis (CE) with a 488 nm Ar-ion laser and multichannel fluorescence detection. The amplified sample was mixed with a dilute solution of the fluorescent intercalating dye, TOTO-1 iodide (514 nm excitation, 533 nm emission) and then allowed to sit for 5 min before being injected into the capillary. Capillaries with an internal diameter of 50 µm and a length of 41 cm (30 cm between the injection inlet and detection zone) were filled with commercial Performance Optimized Polymer 4 (POP-4), consisting of 4% poly(dimethylacrylamide) (PDMA), 8 M urea, 5% 2-pyrrolidinone, and 100 mM N-tris(hydroxymethyl)-methyl-3-aminopropanesulfonic acid (TAPS), at pH 8.0. However, because both poor peak shape and resolution were present when the intercalating dye was added directly to the DNA sample, an alternate procedure was also developed on a Beckman MDQ, which allowed for more accurate allele sizing. On this instrument, a 488 nm laser was utilized and TOTO-1 was added to the separation solutions (sieving matrix and run buffers), not to the sample. The separation system consisted of 7.3% PDMA (MW ~1,000,000) that had been synthesized in house, 8 M urea, 5% 2-pyrrolidinone, and 100 mM TAPS, at pH 8.0. The PDMA synthesis procedure can be found in Appendix A. By comparing the peak positions of the D1S80 ladder, which comprises the most common alleles present in the population for that particular DNA region, to that of the unknown allele(s), the size of the allele(s) were
accurately determined. Detailed descriptions of the DNA extraction, PCR amplification, and CE characterization procedures are given in Appendix B.

2.2. Synthesis and Characterization of λ-DNA with Disulfide Groups on Opposite Strands

2.2.1. Repaired Sample

Nicked and repaired λ-DNA molecules functionalized with disulfide groups at their 3’ ends were synthesized using a hybridization procedure adapted from Braun et al. The 48,502 bp (~16 µm), viral λ-DNA was obtained from Sigma-Aldrich. This molecule possesses a random sequence and its linear form contains a short overhang region of 12 bases in length at each end. These overhang regions were utilized to functionalize the ends of λ-DNA with disulfide groups, allowing for attachment to the Au electrodes used for conductivity measurements. Single strands, 12 bases in length, and labeled with disulfide groups at their 3’ ends via a three carbon linker were synthesized by IDT. These single strands were complementary to the 12 unpaired bases of each overhang region.

To functionalize the overhang regions of the λ-DNA, the 5’ phosphate groups on both ends of the molecule were removed using shrimp alkaline phosphatase. This step was used to prevent the re-formation of circular λ-DNA during the ligation process. The two 12 base-long single strands were phosphorylated using T4 polynucleotide kinase. This enzyme catalyzes the transfer of the γ-phosphate from ATP to the 5’ hydroxyl group of DNA. One of these oligonucleotides was then hybridized to λ-DNA by cooling from
75 °C to room temperature, over a period of 8 h. At this point a gap existed in the phosphate backbone, between the 5’ end of the complementary single strand and the 3’ end of the λ-DNA substrate. This nick was repaired using T4 DNA ligase which catalyzes the formation of phosphodiester bonds between juxtaposed 5’-phosphate and 3’-hydroxyl termini.79 The mixture was then filtered using a Microcon YM-100 device to remove excess free oligonucleotides. Microcon centrifugal filter devices utilize a hydrophilic cellulose membrane with low-adsorption characteristics to concentrate the DNA sample and/or remove small DNA fragments and any salts present.80 Therefore, the YM-100 device could be used to collect DNA fragments with a molecular weight >10,000 Daltons, i.e. a DNA length >125 bp. Following this step, the second oligonucleotide was hybridized and ligated to λ-DNA in the same manner and again, the entire solution was filtered.

To verify that these enzymatic manipulations were working properly, one of the 12 base complementary oligonucleotides was labeled with FAM fluorescent dye. After completion of the final ligation step, the λ-DNA sample was exposed to the restriction enzyme, BglII. Restriction enzymes cut at specific DNA target sequences to yield smaller fragments.78 The BglII-cut λ-DNA was analyzed by denaturing capillary electrophoresis on an ABI 310 Genetic Analyzer. As expected, a single-strand, fluorescent fragment, with a length greater than the 12 base complementary oligonucleotide, was detected (fragment of 419 base length), confirming the realization of successful enzymatic ligations.
2.2.2. Sample with Base Pair Mismatch

A DNA mismatch should disturb base stacking and possibly reduce charge transport efficiency. To explore this idea, a single bp mismatch was introduced into disulfide-labeled λ-DNA. The DNA was synthesized by hybridizing a 12 base oligonucleotide to the λ-DNA template. However, this oligonucleotide was complementary to only eleven of the twelve bases of one of the overhanging arms. A guanine base was switched to an adenine, seven bases over from the 3′-disulfide-labeled terminus. After ligation of this oligonucleotide, a normal, second oligonucleotide that was perfectly complementary to the other arm was hybridized and ligated to the template.

2.2.3. Sample with Two Nicks

Current-voltage characteristics obtained for the repaired λ-DNA were compared to identical λ-DNA samples where the nicks in the phosphate backbones were left unrepaired. These nicked samples were prepared as described above except that T4 DNA ligase was not added to the solution after each of the oligonucleotides was hybridized to the λ-DNA substrate. Instead, after the hybridization of one oligonucleotide the solution was filtered. The second oligonucleotide was then annealed to the remaining λ-DNA arm and after a final filtration, the sample was measured. A detailed description of this procedure and the synthesis of the repaired form of the λ-DNA can be found in Appendix D.
2.3. Synthesis and Characterization of \( \lambda \)-DNA with Disulfide Groups on the Same Strand

2.3.1. Repaired Sample

To synthesize \( \lambda \)-DNA with disulfide groups attached to the same strand, three different single strand oligonucleotides were necessary, in contrast to the synthesis of \( \lambda \)-DNA with disulfide groups attached to opposite strands, as reflected in Figure 2-1. In the first step of this procedure, a 12 base 5′ phosphorylated oligonucleotide, functionalized on the 3′ end with a disulfide group (\( \lambda 2 \)) was hybridized and ligated to the complementary 12-base overhanging end of \( \lambda \)-DNA and the solution was filtered. The second oligonucleotide added to this modified \( \lambda \)-DNA was a 5′ phosphorylated 25 base fragment (\( \lambda \) extension) with part of its sequence complementary to the other 12 base overhanging end of the \( \lambda \) substrate. After the ligation of this oligonucleotide and subsequent filtration, the extra 13 overhanging bases of the \( \lambda \)-extension served as an additional sequence to hybridize a third oligonucleotide to. This final oligonucleotide consisted of a complementary 13 base sequence, functionalized on the 5′ end with a disulfide group attached via a six carbon linker (\( \lambda 3 \)). The hybridization was then followed by a final ligation and filtration.

2.3.2. Sample with Three Nicks

Electrical conductivity measurements were also completed on the DNA sample when three nicks were present. After hybridization of the three complementary oligonucleotides (\( \lambda 2 \), \( \lambda 3 \), and \( \lambda \) extension) to the \( \lambda \)-DNA substrate, the phosphate backbones were left incomplete, i.e. ligation by T4 DNA ligase was not performed. A detailed protocol for the formation of both nicked and repaired \( \lambda \)-DNA with disulfide groups on the same strand can be found in Appendix E.
**Figure 2-1.** Diagram displaying the steps used to form λ-DNA with disulfide groups on the 3′ and 5′ ends of the same strand. Adapted from Reference 72.
2.4. Synthesis of Disulfide-Labeled $\lambda$-DNA with Nineteen Nicks

The effect of having 19 nicks in the phosphate backbones of disulfide-labeled $\lambda$-DNA was also investigated. To synthesize DNA with 19 nicks, $\lambda$-DNA labeled with disulfide groups on the 3’ ends of each strand was mixed with a reaction buffer containing the artificial endonuclease, N.$Bpu$101. This enzyme recognizes a double stranded DNA sequence, 5’ GC$^\wedge$TNAGG 3’, but instead of cutting both strands it nicks the phosphate backbone of only one of the strands at the nineteen recognition sites present in $\lambda$-DNA. Details of this nicking procedure are written in Appendix F.

2.5. DNA Repair and Damage on the Device

After alignment and attachment of DNA strands to the measurement electrodes, repair and damage of the phosphate backbone could also be initiated in situ. This allowed for a direct comparison of the varying structural forms by ensuring that similar quantities of DNA strands were spanning the gap, and that these strands were contacting the electrodes in an equivalent manner.

To repair nicks present in the phosphate backbones, a solution containing the T4 DNA ligase enzyme and its corresponding ligation buffer was added to the device. The device was then placed in a sealed fluoroware container to prevent droplet evaporation and left at room temperature for at least 6 h (the optimal ligation temperature is 22 °C).

After alignment/attachment of an undamaged $\lambda$-DNA sample on the device, nineteen nicks were created in its backbone by adding a solution containing the N.$Bpu$101 enzyme and its corresponding reaction buffer. Again, the size of the droplet
was sufficient to cover the entire gap and the device was placed in a sealed fluoroware container. The active temperature of this enzyme is 37 °C and therefore, the container was placed in an appropriately heated oven for 1 h. Detailed procedures for the repair and nicking of λ-DNA on the devices are given in Appendices F and G.


By utilizing the labeling method developed for the addition of disulfide groups to the 5′ and 3′ ends of the same DNA strand (Section 2.3.1), labeled single stranded λ-DNA was formed. This allowed for direct comparison of charge transport in ssλ-DNA to transport in dsλ-DNA. The ssλ-DNA was formed from the dsλ-DNA using two different methods for comparison purposes.

2.6.1. Enzymatic Digestion

In the first method of ssλ-DNA formation, the unlabeled strand was removed using an enzymatic reaction. λ-exonuclease selectively digests the strand of the duplex containing a 5′ phosphate, in a 5′ to 3′ direction. Therefore, the complementary strands of the disulfide-labeled duplex, which contained a 5′ phosphate were degraded into mononucleotides when exposed to this enzyme in its reaction buffer, while heating the solution at 37 °C for 2 h. These free mononucleotides could then be removed during device washing, prior to measurement.

2.6.2. Chemical/Thermal Denaturation

In the second method, a solution of the disulfide-labeled λ-DNA was heated to 95 °C for 5 min to thermally disrupt the hydrogen bonds between the base pairs, causing the
two strands to dissociate. This process was further encouraged using a denaturant, formamide, which destabilizes the duplex by chemically disrupting hydrogen bonding. After heating, the solution was snap-cooled on ice to prevent re-hybridization. Again, the unlabeled strand could be rinsed away after the alignment and attachment of the labeled strand.

In order to make ssλ-DNA measurements that were quantitatively comparable to the dsλ-DNA measurements, the samples that were to become single stranded were initially at a concentration that was twice that of the samples that were measured in their double stranded form. This concentration difference was necessary because once the dsλ-DNA sample was exposed to λ-exonuclease or thermally denatured, only the strand with the disulfide labels would be anchored to the gold electrodes, the other strand or its fragmented remains would be removed prior to measurement. Details of these two ssλ-DNA protocols can be found in Appendix H.

2.6.3. Formation of Single Stranded λ-DNA on the Device

Both of these methods for ssλ-DNA formation could also be performed on the device after the alignment and attachment of the disulfide-labeled dsλ-DNA sample. To generate ssλ-DNA, solutions of λ-exonuclease with its corresponding reaction buffer or formamide were added to the devices in sufficient volumes to cover the electrode gaps. The devices were then placed in sealed fluoroware containers and heated at either 37 °C for 2 h for the enzymatic reaction or 95 °C for 5 min, followed by a snap-cooling step on ice for the thermal/chemical denaturation.
2.7. Synthesis and Characterization of Short λ-DNA

To gain a better understanding of the effect of strand length on DNA conductivity a 7460 bp DNA fragment containing a random sequence was examined. This fragment was created by digesting λ-DNA with a restriction enzyme.

2.7.1. BplI Digestion

To prepare disulfide-labeled λ-DNA fragments with lengths of 7460 bp (~2.5 µm) the restriction enzyme, BplI was used. The starting material (16 µm λ-DNA) was mixed with BplI enzyme, its corresponding reaction buffer, and S-adenosylmethionine (SAM) and this solution was incubated at 65 °C for 20 min. BplI requires only Mg²⁺ for its activity, which is present in its reaction buffer but it is also known to be stimulated by SAM. Only one recognition sequence was present on the λ-DNA molecule,

5′…↓₈(N)GAG(N)₃CTC(N)₁₃↓…3′

3′…↑₁₃(N)CTC(N)₃GAG(N)₈↑…5′

at a distance of 41,042 bp and therefore, exposure of the full length λ-DNA to this enzyme yielded a 7460 bp fragment. This short fragment had a 5 base long overhanging region where the DNA had been cut, in addition to the molecule’s natural 12 base long arm on its opposite end.

2.7.2. Slab Gel Electrophoresis

To determine the success of this digestion, slab gel electrophoresis was utilized for separation and detection of the product sizes. Although shortened, the λ-DNA fragment was still too large for CE analysis. A 1% agarose gel was prepared in
tris(hydroxymethyl)aminomethane (Tris)-borate-ethylenediaminetetraacetic acid (EDTA) (TBE) buffer and the DNA fragments were detected using ethidium bromide dye and UV irradiation. Fragment sizes were compared to an external standard, a 1 kb DNA ladder.

2.7.3 Disulfide Labeling

To label the short λ-DNA fragment it was mixed with a phosphorylated 12 base oligonucleotide, complementary to its natural overhang region. This oligonucleotide was functionalized with a 3' disulfide group through a three carbon linker. Following the hybridization and ligation of this strand, the solution was filtered and then mixed with a 5’ disulfide-labeled, 5 base oligonucleotide that was complementary to its restriction enzyme cut end. After the hybridization and ligation of this second oligonucleotide, the solution was again filtered. An in depth procedure for the formation, characterization, and labeling of this DNA sample is located in Appendix I.

2.8. DNA Quantitation

To obtain a more accurate estimate of how many DNA strands were being measured on each device, a DNA quantitation procedure was developed. This method could be used to determine the amount of disulfide-labeled λ-DNA that was present in a solution before an aliquot of that solution was added to a device. Two DNA calibration curves covering the ranges, 20 ng-1000 ng and 200 ng-3 µg, were created using bisbenzimide dye, calf thymus DNA standards, and a Perkin Elmer LS 45 Spectrometer. The calibration curve for the lower range is shown in Figure 2-2. Bisbenzimide, commonly known as Hoechst 33258 dye, is a fluorescent dye that binds to the minor
**Figure 2-2.** DNA calibration curve established using a calf thymus DNA quantitation standard and Hoechst 33258 dye. Each point represents the average of three fluorescence measurements for that particular quantity. The fluorescence intensities were measured in relative fluorescence units (RFU).
groove of DNA. This dye is excited by 360 nm light and its fluorescence at 460 nm can be measured. After the creation of these standard curves, the amount of λ-DNA that was successfully labeled with disulfide groups could be estimated by measuring the fluorescence of the solution before and after the addition of a gold-coated strip. This strip was of constant size (25 mm × 2 mm) and was allowed to remain in the solution for 1 h before an aliquot was taken and the fluorescence measured. Using these estimates, different DNA batches could be diluted appropriately so that the initial concentration added to each device was consistently 5 nM. By keeping the concentration constant, direct comparisons could be made among the conductivity measurements obtained for different DNA samples. A comprehensive protocol for DNA quantitation can be found in Appendix J.

2.9. Device Fabrication

Devices were fabricated in Dr. Jean Heremans’ lab by Daniel Asare, Forrest Bradbury, and Drs. Hong Chen, Jean Heremans, and Victoria Soghomonian.

2.9.1. 120 and 180 nm Gaps

The electrodes used for the measurement of the D1S80 samples were created using electron beam lithography. After writing the desired pattern on poly(methyl methacrylate)-coated substrates consisting of p-type-doped Si (100) capped with a 4500 Å thermal oxide, the damaged polymer was rinsed away. Electron beam evaporation was then used to deposit an 80 Å Cr sticking layer, followed by a 300 Å Au layer. The electrodes were spaced approximately 120 or 180 nm apart and run parallel over a length
of about 5 μm, Figure 2-3 (a). Following fabrication of the electrode gap, standard photolithography was utilized to create larger gold pads around the electron beam device. AZ5206E photoresist was spun on the SiO₂ to form a 0.6 μm layer. After baking at 95 °C, the photoresist was exposed through a mask to UV radiation. The pattern was developed using a standard alkaline developer for 20 s and then electron beam evaporation was again used to evaporate an 80 Å Cr layer and a 300 Å Au layer. The final step in this device fabrication was an acetone lift-off to remove remaining photoresist.

2.9.2. 1.2 and 8 μm Gaps Fabricated Using Photolithography

Gold electrodes used for the measurement of the various forms of disulfide labeled λ-DNA were fabricated using standard photolithography and lift-off, as described above. Two different masks were used to create the different gap sizes (1.2 μm and 8 μm). The device substrates consisted of p-type-doped Si (100) capped with a 4500 Å layer of thermal oxide. After photolithography an 80 Å Cr layer was deposited on the substrate using electron-beam evaporation, followed by a 300 Å Au layer. The electrodes that were separated by 8 μm, run parallel over a length of 50 μm, allowing for a large number of DNA molecules to span the gap, Figure 2-3 (b). The electrodes that were separated by 1.2 μm, run parallel over a length of 7.5 μm, Figure 2-3 (c). It was found that careful control of the lift-off profile was necessary in order to avoid upturned electrode edges, which prevented DNA strands from spanning the gaps.
Figure 2-3. The Au electrode geometry on Si/SiO$_2$ substrates. (a) SEM images of device with a 180 nm gap, at different magnifications. (b) Optical image of a device with an 8 µm gap. (c) Optical image of device with a 1.2 µm gap.
2.9.3. Gaps Fabricated Using Tungsten Wires

An alternative method for device fabrication involved the use of wires to create electrode gaps. In this technique, commercially available tungsten wires of 15 and 7 µm were stretched and attached across mica or SiO₂ substrates. After gold deposition using electron-beam evaporation, devices with gap sizes <15 µm were created by removal of the wires.

2.10. Device Cleaning

A rigorous cleaning of the electrodes prior to DNA attachment was necessary to obtain reproducible results with disulfide-labeled λ-DNA. This cleaning procedure involved rinsing the device for 30 s in a 1:1:5 (v/v) solution of ammonium hydroxide/hydrogen peroxide/deionized water at 75 °C to remove organic residues, 10 min in acetone at 65 °C, 10 min in methylene chloride at 55 °C, and finally, deionized water. Devices were then dried thoroughly prior to measurement using a flow of nitrogen gas. These steps led to abundant gold binding sites, facilitating the formation of many chemisorbed disulfide-Au bonds, and hence, adequate electrical contacts.

2.11. Alignment and Attachment of DNA

Alignment and attachment of the various forms of disulfide-labeled λ-DNA to Au electrodes separated by 8 µm has also been optimized so that reproducible conductivities can be measured. In the procedure, DNA was aligned between the Au electrodes using ac electric fields with a strength of 10⁶ V/m and a frequency of 1 MHz (applied using a Hewlett Packard 33120A function generator and monitored using a Tektronix TDS 3054 Oscilloscope).⁸⁸,⁸⁹ With the ac voltage applied to the electrodes, a 2 µL drop of 5 nM
DNA solution was centered over the electrodes. During the 25 min alignment period, the sample was placed within a hydration chamber where the relative humidity (RH) was kept above 90% to control droplet evaporation. After removing the electric field, the device was left for an additional 25 min in the chamber. Finally, the alignment step was followed by a rinse with deionized water to remove both unbound DNA and any salts present and the sample was dried under a flow of nitrogen gas.

To investigate whether the alignment procedure was successful, confocal microscopy was utilized with the DNA fluorescent intercalating dye, TOTO-1-iodide. After the alignment of the DNA strands on the device, a solution of this dye was added and allowed to bind to the DNA for approximately 15 min before rinsing the device with deionized water. A confocal microscope with an Ar-ion laser was then used to determine the position of the DNA and/or the approximate number of strands attached to the electrodes.

2.12. Current-Voltage Measurements and Data Collection

$I-V$ characteristics of the various DNA samples were examined at ambient conditions using a Keithley 2400 SourceMeter, a Stanford Research Systems SR570 Low-noise Current Preamplifier, and a Keithley 2000 MultiMeter. At the start of each day of measurements, the $I-V$ curve for a 100 MΩ resistor was collected using a potential sweep from -0.5 V to 0.5 V, to check that all instruments were functioning correctly. Data was acquired using a program written in National Instruments LabView™ 5.1. In addition, prior to measuring the $I-V$ characteristic of a particular DNA sample, an air gap of that device (the device without DNA, after cleaning) was always taken using a
potential sweep from -20 V to 20 V. These air gaps were used to confirm the absence of a short circuit and the lack of SiO₂ breakdown, which typically only occurs with fields >10⁷ V/m. DNA data was collected by starting with a low potential sweep, -0.5 V to 0.5 V (followed by a sweep with the same range but in the reverse direction) and then the sweep was gradually increased up to ±20 V. A step voltage of 25 mV was used for ±0.5 V sweeps, 250 mV for ±5.0 V, 500 mV for ±10 V, and 500 mV for ±20 V. The sweep parameters also included a soak of 10 seconds after the voltage ramp, prior to data collection and a wait of 0.10 seconds between data points.

2.13. DNA Fiber Formation and Positioning on Electrodes

To gain a better understanding of the relative contributions of other components besides the π-stack to DNA’s electronic properties, macroscopic DNA fibers were measured. An alignment procedure was not utilized to orient the individual strands of these fibers across the electrode gaps and therefore, it is unlikely that strands were stretched with one end on one electrode and the other end on the second electrode. Instead, charge transport measured through these fibers can be attributed to interstrand DNA conduction and ionic/water conduction along the phosphate backbone.

Fibrous DNA bundles were formed using a procedure adapted from Mitsui et al.⁹¹ Prior to use, calf thymus DNA from Sigma was sized using slab gel electrophoresis and the majority of the DNA was found to range in length between 10,000 and 50,000 bp. To form DNA fibers, this DNA was diluted in Tris-EDTA buffer to a concentration of 5 mg/mL and allowed to stir overnight to completely dissolve into solution. An aliquot of the DNA solution was then placed in a beaker and ice cold 95% ethanol was gradually
added down its side, while slowly stirring the solution with a magnetic stir bar. Ethanol was added until no new precipitate was formed between the two solvent layers. The fibrous precipitate was then picked out from this interface using tweezers. A second pair of tweezers was used to hold onto the other end of the bundle and then it was lightly pressed against filter paper to remove excess water. The fiber was gently stretched by continually applying tension as it dried. Some fibers could be stretched to >15 mm in length, while the fiber widths ranged from 10-250 µm.

For conductivity measurements, the fibrous bundles were contacted in one of two ways. The ends of the fiber were contacted with indium or the fiber was manually aligned across various gold electrode gaps and held in place with a mechanically pressed compliant layer.

### 2.14. Ion Exchange Methods

Initially, an aggressive ion-exchange method was used to manipulate the DNA counterions to determine the effect of their identity on the resulting conductivity measurement. This involved mixing 10 µL of 5 nM disulfide-labeled λ-DNA (with the functional groups on the 3’ ends of opposite strands) with 10 µL of 100 mM LiCl, RbCl, CsCl, KCl, NaCl, CaCl₂, MgCl₂, NiCl₂, ZnCl₂, CoCl₂, AlCl₃, or FeCl₃ in 5 mM Tris-HCl buffer at pH 7. After mixing, the solutions were allowed to sit at ambient conditions for 12 h. Excess salts, not bound to the DNA strands were then removed from the solutions using Microcon YM-100 filters. Finally, the retentate from each of these filtrations was diluted with deionized water to a concentration of 5 nM for I-V measurement.
The second, less aggressive ion-exchange method utilized Millipore “V” series membrane filters for drop dialysis. With this method a floating cellulose filter disc (13 mm diameter) containing a membrane with a 0.025 μm pore size removes or exchanges salts via reverse osmosis. The procedure involved first floating the membrane filter disc on the surface of the dialysis buffer (containing 100 mM of the desired cation in 5 mM Tris-HCl, pH 7) in a petri dish. 10 μL of 5 nM disulfide-labeled λ-DNA was then deposited on the center of the membrane and the dish was covered to minimize loss of sample by evaporation. After 1 h at ambient conditions the DNA sample was removed from the disc using a micropipette. Finally, Microcon YM-100 filters were used to remove some of the excess salts present in the dialyzed DNA sample solutions and the retentates from these filtrations were diluted with deionized water to 5 nM DNA concentrations.

2.15. Imaging DNA with Atomic Force Microscopy

Atomic force microscopy (AFM) can be used to image the surface of samples on a nanometer scale and because of its high resolution, this technique has often been applied to studying the morphologies of biological molecules. In the current studies AFM was used to directly visualize various forms of chemically-manipulated DNA molecules and also to observe the alignment of DNA strands across electrode gaps. AFM images were collected using a Thermomicroscopes Explorer equipped with a 100 μm Tripod scanner and operating in non-contact mode. All of the images were taken using high resonance frequency silicon probes from Veeco.
DNA samples were typically imaged on mica substrates or after ac alignment, on SiO$_2$ devices. Mica is ideal for imaging because it is atomically flat over a large area. However, due to its significant negative charge density (one charge per $\sim$0.47 nm$^2$), negatively charged DNA molecules do not adsorb on bare mica to any appreciable extent.$^{95,96}$ To assist the sample adsorption process, MgCl$_2$ can be added either to the DNA sample solution or to the mica prior to adding the sample.$^{94}$ The addition of this salt allowed the biomolecules to bind strongly enough to the surface that the disc could be rinsed with deionized water after deposition.

To prepare a B-DNA sample for imaging, a 10 $\mu$L droplet of 0.1 nM $\lambda$-DNA in a solution of 10 mM MgCl$_2$, 10 mM Tris-HCl buffer, pH 8.3 was deposited onto a freshly cleaved mica surface. To cleave the substrate a piece of scotch tape was used to pull off a few of the top mica layers. After a 10 min incubation at ambient conditions to allow for DNA adsorption, the disc was rinsed with copious amounts of deionized water to remove buffer salts. The surface of the mica disc was subsequently blown dry using a flow of nitrogen gas.

To prepare a counterion-exchanged DNA sample for imaging, the mica was instead treated with a 10 $\mu$L drop of 10 mM MgCl$_2$ prior to the addition of this sample to the substrate. The solution was left on the mica for 10 min and then the surface was rinsed and dried, and the DNA sample was added in the same manner.

**2.16. Metallated DNA Formation**

Previous experiments have shown that the imino protons of the DNA base pairs can be replaced with certain transition metal cations to form metallated DNA (M-
This complex is known to form in the presence of Zn\(^{2+}\), Ni\(^{2+}\), and Co\(^{2+}\) when the solution’s pH is above 8.

### 2.16.1. Samples for Fluorimetry

A Perkin Elmer LS 45 Luminescence Spectrometer was used to measure the extent of covalent binding of different metal cations to the nucleobases. The technique was also used to measure the effect of pH on this metal/DNA interaction, the length of time necessary for binding, and the stability of the M-DNA once formed. It has previously been shown that when metal ions are incorporated into the DNA helix, the intercalating dye, ethidium bromide, cannot bind to the DNA because of charge repulsion.\(^97\) This process results in the fluorescence intensity of a sample being much lower than expected for its particular DNA concentration, with reduction proportional to the extent of M-DNA formation. To perform a similar fluorescence assay in this dissertation work, a different intercalating dye, TOTO-1-iodide was used. This dye was also found to be excluded from binding to DNA base pairs because of metal incorporation. The assay solutions consisted of 0.2 µg of λ-DNA added to 150 µL of buffer containing 10 mM Tris-HCl, pH 8.3 or pH 7 and various concentrations of ZnCl\(_2\), NiCl\(_2\), CoCl\(_2\), and FeCl\(_2\). Once mixed, each solution was allowed to interact for 1 h, with the exception of the time and stability studies where aliquots were taken at variable times. As a control, the same amount of DNA was also added to 150 µL of 10 mM Tris-HCl, pH 8.3 without the metal cations present. After the allotted time, 0.2 µL of TOTO-1 (0.25 mM) was added to the solution, mixed and allowed to interact for 5 min. Each solution was then pipetted into a microcuvette and the fluorescence intensity was
measured at ~533 nm (excitation 488 nm). The relative fluorescence intensity (%) was calculated by dividing the intensity of the test DNA sample by the intensity of the DNA control, i.e. a solution containing the same concentration of DNA in a Tris-HCl buffer with TOTO-1 but no metal cations added.

Because DNA fragments labeled with fluorescent dyes were examined in the CE studies, a LS 45 Spectrometer was also used to determine the ability of the various metal ions to fluorescently quench the dye-labeled DNA samples. This was done by mixing the multiply dye-labeled allelic ladder, Profiler Plus or the fluorescein-labeled TH01 allele 9.3 with the metal cations.

2.16.2. Samples for Capillary Electrophoresis

To metallate the molecules for CE, a dye-labeled DNA sample (DNA ladder or PCR amplified product) was placed in 0.5 mM-100 mM NiCl₂, ZnCl₂, or CoCl₂ in a 10 mM Tris-HCl buffer, pH 8.3. The solutions were vortexed to mix evenly and then centrifuged. The samples were allowed to interact for at least 1 h under ambient conditions. For controls, these same DNA fragments were also mixed in Ni and Co buffer solutions at the same concentrations but at pH 7 and in buffers at pH 8.3 but with cations such as Mg²⁺ and Fe³⁺, which do not intercalate and covalently bind to the DNA bases. Typically, a 1 μL aliquot of each of these solutions and 0.5 μL of ROX-labeled DNA (internal size standard) were added to 11 μL formamide. For denaturation, samples were heated at 95 °C for 2 min and snap-cooled on ice.

CE with multi-channel laser induced fluorescence (LIF) detection was accomplished using an ABI Prism 310 Genetic Analyzer. Capillary columns with an
internal diameter of 50 µm and a length of 43 cm were filled with POP-4. Samples were electrokinetically injected using 15 kV for 5s and the fragments were separated for 24 min at 15 kV and 60 °C. It was found that the data processing software used by an ABI 310 to perform color separation caused a reduction in the intensity of some of the very broad peaks or shoulders just visible in the raw data. For this reason some of the raw data was examined rather than data that had been analyzed using the GeneScan software (Version 3.1). The 250-base fragment from the GeneScan-500 ROX was not included in the generation of size standard curves due to its tendency to migrate anomalously under non-ideal conditions on an ABI 310.

2.16.3. Samples for Atomic Force Microscopy

For AFM measurements, 0.2 µg of λ-DNA was added to 150 µL of buffer containing 10 mM Tris-HCl, pH 8.3 or pH 7 and 3.0 mM NiCl₂, 3.0 mM CoCl₂, or 20 mM ZnCl₂. After mixing, the solutions were left for 1 h at ambient conditions and then aliquots were added to mica substrates. The presence of these transition metal salts allowed the DNA to bind tightly enough to the negatively charged mica that the addition of MgCl₂ to the solution or the substrate surface was not necessary. After preparation, the samples were immediately imaged.

2.17. Repetitive DNA Synthesis, Characterization, and Collection

Preliminary theoretical and experimental work has shown that more repetitive DNA sequences appear to support charge transport in this biomolecule to a greater extent. Therefore, methods were developed to enzymatically ligate commercially
available short fragments of repetitive DNA to form molecules long enough to span the electrode gap sizes that can be fabricated.

2.17.1. Ligation

To prepare ligated, double stranded, repetitive DNA sequences without gaps or overhanging ends, the following procedure was used. First the commercially available, single stranded, repetitive DNA starting material: 20 base poly(G), 20 base poly(C), 40 base poly(A), 40 base poly(T), or 30 base poly(AT), was phosphorylated on its 5′ end using T4 polynucleotide kinase. This phosphorylated, single stranded DNA was then hybridized to its complementary, phosphorylated strand or with the case of the poly(AT), it was hybridized to itself to form duplex DNA. Hybridization was performed by heating a solution containing the complementary strands to 90 °C and then slowly cooling the solution to room temperature using the GeneAmp® 9700 or a water bath. The imperfectly hybridized starting material (imperfect due to the highly repetitive nature of the DNA) was then made blunt-ended utilizing the 5′ to 3′ synthesis activity/3′ to 5′ exonuclease activity of T4 DNA polymerase. Finally, this blunt-ended product was linked end-to-end using T4 DNA ligase, in the presence or absence of 50% (w/v) polyethylene glycol (PEG) 4000. T4 DNA ligase is able to join two dsDNAs (blunt-end or sticky-end ligation) or seal a nick between two ssDNA fragments annealed on the complementary strand, as described in Section 2.2.1. By using PEG in the reaction mixture, it was found that both the ligase activity and the ligation efficiency of certain fragment lengths could be improved. Details of this procedure can be viewed in Appendix K.1.
2.17.2. Thiol Labeling

Ligated repetitive sequences were labeled with thiol groups for attachment to the gold electrodes utilized in \( I-V \) measurements. To accomplish this, the mixture of repetitive DNA fragments was first added to a reaction buffer containing alkaline phosphatase. This enzyme removes the 5' phosphate groups on the DNA and replaces them with 5' hydroxyl groups. For optimal enzyme activity, the solution was heated at 37 °C for 30 min. The dephosphorylated DNA was added to a second reaction mixture, which included ATP\(_\gamma\)S and T4 polynucleotide kinase. This solution was heated at 37 °C for 30 min, activating the kinase to transfer thiophosphates from the labeled ATPs to the 5' hydroxyl groups of the nucleic acids. Labeling was accomplished prior to separation and collection of the fragments using high performance liquid chromatography (HPLC) because ammonium ion concentrations greater than 5 mM are known to inhibit polynucleotide kinase activity, decreasing labeling efficiency.\(^{103}\) In the HPLC separation utilized, 100 mM triethylammonium acetate was present in the mobile phase. A complete protocol for 5' end labeling is located in Appendix K.4.

2.17.3. CE Characterization

CE was used to characterize the lengths of the various fragments present in the repetitive DNA ligation mixtures. The initial sieving matrix employed for this dsDNA separation was hydroxyethylcellulose (HEC). However, after the development of a procedure using poly(vinylpyrrolidone) (PVP), this polymer was used for all further DNA characterization.
In electrophoresis, different molecules are usually separated as a result of their different mobilities. However, since DNA molecules have a constant charge/size ratio, their electrophoretic mobility in free-solution is equal regardless of their chain length.\textsuperscript{104} For this reason, a sieving matrix must be used within the capillary to provide size-dependent DNA fragment separation.

Initial separations with HEC (MW 250,000) involved using a 2\% solution of this polymer in TBE with TO-PRO-3 intercalating dye (642 nm excitation, 661 nm emission) or YO-PRO-1 intercalating dye (491 nm excitation, 509 nm emission) added to the buffer and sieving matrix. Depending on the dye used, a 635 nm diode laser or a 488 nm Ar-ion laser were used for LIF detection on a P/ACE 2050 CE system. Details of this separation system can be found in Appendix K.2. While the separation performance was high using this HEC system, expensive fluorocarbon-coated capillaries had to be used to reduce electroosmotic flow (EOF) because of this polymer’s poor coating ability. Polymers with a high dynamic coating ability reduce the EOF without the need for a capillary coating. In this sense, PVP is attractive.\textsuperscript{105,106} Its coating ability is thought to result from hydrogen bonding between the PVP carbonyl groups and the capillary wall. Furthermore, it has a low viscosity compared to other polymers at the same concentration and molecular weight and therefore, does not require as high of pressure or as long of time to be loaded into the capillary. In addition to these properties, a solution of PVP has been chosen for dsDNA separation because of its short preparation time (the HEC solution must be stirred overnight and filtered prior to use) and long shelf life.
The CE procedure utilized for DNA separation was adapted from Gao and Yeung. A solution of 5.5% PVP (MW 1,000,000) in TBE was used in a capillary with a 50 µm internal diameter and a length of 37 cm. Separation was performed on a P/ACE 2050 system with a 488 nm Ar-ion laser and YO-PRO-1 intercalating dye utilized for detection. The DNA sample was electrokinetically injected and separated using a run voltage of 5 kV (i.e. an electric field of 135 V/cm). Between runs the capillary was regenerated using a deionized water rinse, followed by a methanol rinse. PVP is readily soluble in both of these solvents. With this procedure, greater than 8 bp resolution for DNA fragments ranging in size from 60 to 600 bp, could be achieved. A more thorough description of this method can be viewed in Appendix K.3.

2.17.4. High Performance Liquid Chromatography and Fraction Collection

After enzymatic ligation of the repetitive sequences, multiple fragment sizes were present in the reaction mixture. Therefore, a separation and collection technique had to be utilized if only one length of repetitive DNA was to be measured. For this purpose a bioinert HPLC system was set up with a Hitachi L-6220 manual injector and pump. This system was connected to an Applied Biosystems 783 absorbance detector and a Gilson FC 203B fraction collector. An Agilent Eclipse DNA column (4.6 mm ID x 75 mm), consisting of a wide poor silica support with a chemically bonded aliphatic organosilane stationary phase was used to perform the separation. This non-polar stationary phase achieves high resolution separations of double stranded DNA fragments using an ion-pairing mechanism. The cationic ion pairing agent used with the column was triethylammonium acetate (TEAA) and separation was conducted using a gradient elution
technique. Longer fragments of DNA contain more phosphate groups and therefore, the number of ion-pairing sites are directly determined by the length of the DNA. For this reason, longer fragments elute later than shorter fragments.

The mobile phase consisted of two components, A) 0.1 M TEAA/0.1 mM EDTA (pH 7.0) and B) 0.1 M TEAA/0.1 mM EDTA (pH 7.0) in 25% acetonitrile. EDTA was added to the mobile phase buffers to remove polyvalent metal cations in the HPLC system and increase separation performance. Column equilibration with 40% eluent B for approximately 2 h prior to sample injection was found to be necessary after any long term column storage. The gradient range employed for separation was 40-80% eluent B in 30 min at 45 °C with UV absorbance at 260 nm utilized for detection.
Chapter 3. Manipulating DNA for I-V Measurements

3.1. DNA Attachment

Over the past decade there has been a great deal of interest in techniques for the manipulation and attachment of DNA molecules to surfaces. Due to the strong affinity of thiols to gold surfaces, this chemistry is widely employed when attaching DNA to substrates. Thiols, alkylthiols, and dialkyldisulfides are all known to bind to gold with high affinity. The consensus regarding thiol (SH) chemisorption is that the hydrogen atoms are dissociated upon adsorption to the surface. The mechanism of disulfide (SS) chemisorption is thought to result from a simple oxidative addition of the SS bond to the gold surface:

\[
\text{RSSR} + \text{Au}_n^0 \rightarrow 2 \text{RS}^- \text{Au}^+ + \text{Au}_{n-2}^0
\]

Once formed, Au thiolates are known to be quite stable with heats of adsorption of -44 kcal/mol.

The standard method of functionalizing DNA strands with thiols is to attach a HS(CH$_2$)$_x$ or (CH$_2$)$_x$SS(CH$_2$)$_x$ linker to either the 3’ or the 5’ end of the molecule. It has been reported that chemisorption of thiolated ssDNA to a clean gold surface results in a surface coverage of about $10^{13}$ molecules/cm$^2$ or about 1 strand per 10 nm$^2$.

3.2. DNA Alignment

Positioning and stretching of DNA molecules has been demonstrated using a variety of methods including fluid flow, moving meniscus, optical force, magnetic beads, and dielectrophoresis. Dielectrophoresis has been extensively used for trapping and
manipulating bacteria and blood cells, but has only recently gained attention for use in manipulations of DNA molecules on microfabricated structures. The physical basis of dielectrophoresis is that in a non-uniform electric field, polarizable objects experience forces that lead to translational motion. The dielectrophoretic force is directed towards the region of strongest field strength and is a function of the magnitude and frequency of the electric field, the dielectric properties of the medium and the object, and the volume of the object. For example, smaller molecules that are less polarizable, require larger dielectrophoretic forces for orientation.

DNA is a polyelectrolyte and in solution the phosphate groups on the DNA sugar-phosphate backbones dissociate to yield a negatively charged molecule surrounded by positively charged counterions, some condensed on the molecule and some surrounding it in a diffuse cloud. These ions are bound axially but are free to move radially. Therefore, DNA is highly polarizable and a dipole, $p$, is induced in this molecule when an electric field, $E$, is applied, and is given by $p = \alpha VE$, where $\alpha$ is the polarizability of the molecule per unit volume and $V$ is the volume of the molecule. The field not only leads to the movement of DNA, but also to the alignment of the molecules parallel to the electric field and their extension from random coil conformations.

The electric fields that are typically used when dielectrophoresis is performed in solution are ac to avoid the electrochemistry and electrophoresis that can occur with dc fields. These ac fields commonly range from 100 kHz to 5 MHz. The higher the frequency, the larger the voltage that can be applied without the occurrence of
electrochemical reactions. However, the use of too high a frequency can cause a decrease in the induced dipole, due to the reorientation of the dipole on the DNA lagging behind the switch in the polarity of the electrodes.

3.3. Results with Disulfide-Labeled λ-DNA (48,502 bp)

In this study, commercially synthesized oligonucleotides with disulfide functional groups were bound to linearized λ-DNA and used without further modification. The rigorous cleaning procedure described in Section 2.10 was utilized to maximize adsorption of disulfide-labeled DNA. The effectiveness of this procedure was confirmed using confocal microscopy. Different cleaning procedures were compared to determine which method allowed for the greatest fluorescence intensity, i.e. the largest quantity of DNA, to remain on the Au surface after copious rinsing with deionized water.

3.3.1. Characterization of Labeling Efficiency and Test of Disulfide-Au Stability

Figure 3-1 displays the results of a quantitative analysis of disulfide-labeled λ-DNA using the Hoechst 33258 dye. By using this dye, which binds to the minor groove of DNA, it is possible to estimate the concentration of DNA from the fluorescence intensity measured. In addition, this procedure can be used to estimate the amount of DNA that is labeled with disulfide groups. As shown in Figure 3-1 (a), it was found that after adding and then removing a Au-coated strip from unlabeled DNA there was only a very slight reduction (~3 %) in the fluorescence intensity of that solution, demonstrating minimal adsorption. Alternatively, after adding the Au-coated strip to DNA with disulfide functional groups, the fluorescence intensity was lowered by ~75 %, Figure 3-1.
Figure 3-1. Representative fluorimetry results using Hoechst 33258 dye for characterization of disulfide-labeling efficiency. (a) Fluorescence measurements before and after the addition of a Au-coated strip to unlabeled $\lambda$-DNA. (b) Fluorescence measurements before and after the addition of a Au-coated strip to disulfide-labeled $\lambda$-DNA.
(b). This demonstrated that the labeled λ-DNA was attached to Au via the disulfide group. Furthermore, using this procedure ensured that the different batches of functionalized λ-DNA had approximately the same number of successfully labeled strands introduced to the electrode gap of each device. Therefore, comparisons of the $I-V$ curves could be made among the different DNA samples.

The stability of the immobilized DNA on the electrodes was estimated using atomic force microscopy. Double stranded or single stranded disulfide-labeled λ-DNA bound to a gold circle (50 µm diameter) deposited on mica were imaged after repeated deionized water rinses. The DNA solutions were diluted to a concentration that yielded approximately 50 strands attached to each gold pad. Therefore, the number of strands within a 50 µm AFM scan range could be individually counted. Even after vigorous washing ($5 \times \sim 10$ mL), almost all of the DNA that was originally on the gold was still attached via its end labels.

3.3.2. Alignment Across 8 µm Gaps

On the devices, the highest field strength occurs at the electrode gap and therefore, the polarized DNA is attracted to this region. To orient the disulfide-labeled λ-DNA molecules, Au electrodes separated by approximately 8 µm were utilized. Gap sizes were half the length of fully stretched DNA so that even if strands were not completely extended by the field used, they would still be able to span the electrodes. An electric field of $10^6$ V/m and a frequency of 1 MHz was used to orient the DNA on the device. These were the optimized parameters experimentally determined by Washizu and
Kurosawa for λ-DNA and proved to align large quantities of DNA in these experiments. In addition to these parameters, after applying the DNA solution to the electrode gap, the device was immediately placed within a hydration chamber to control droplet evaporation during the 25 min alignment period. Furthermore, after removing the aligning field, the sample was left for an additional 25 min in the chamber to allow for sufficient Au-S bond formation, before rinsing the device. It was found that these long alignment and bond formation times, under controlled humidity, were necessary to obtain reproducible trapping of the DNA.

Success of the ac method of alignment could be visually confirmed using confocal microscopy and TOTO-1 fluorescent intercalating dye as shown in Figure 3-2 (a). This device had an evenly distributed amount of fluorescence intensity both on the inside edges of the gap and within the gap. This technique could also be used to determine the reason for low measured current values when problems were occurring with a particular batch of DNA or devices. Figure 3-2 (b) displays a confocal image of a device that gave an $I-V$ curve that was not much larger than its air gap. From the image, it is clear that not many DNA strands are stretched across the gap and attached to both electrodes. Instead, the majority of the strands are attached to the edge of the right electrode, possibly due to the other electrode being damaged or contaminated with photoresist.

Confocal microscopy could only be used to display the presence or absence of DNA molecules in the electrode gaps. To gain a better idea of the orientation of these strands when they were aligned and the approximate quantity attached to a particular device, an analysis by AFM was attempted. However, because of the
Figure 3-2. Confocal images of devices with an 8 µm electrode gap geometry. DNA imaged using TOTO-1 fluorescent intercalating dye and an Ar-ion laser. (a) Device that gave substantial current values with applied potential sweeps. (b) Device that yielded an $I$-$V$ curve that was just above its air gap measurement.
roughness of the SiO$_2$ substrate used for device fabrication, estimated at ± 2 nm by AFM, DNA strands could not be imaged. Instead, an 8 µm Au electrode gap was fabricated on the atomically flat mica substrate as described in Section 2.9.1. On this surface, disulfide-labeled λ-DNA could be imaged after alignment with an ac field as shown in Figure 3-3. Numerous, aligned DNA bundles containing large numbers of individual strands were found to span the gap. Unfortunately, several areas of the Au electrodes lifted off the mica during the final rinse step, causing the shorting of the device and making it impossible to measure the conductivity of the immobilized DNA.

As for possible damage induced by the application of an electric field to DNA, Heaton _et al._ have reported that a dc field of $10^9$ V/m is needed to denature surface immobilized 25 bp DNA duplexes with mismatches.$^{121}$ Therefore, an ac field strength of $10^6$ V/m should be well below the value necessary to break the hydrogen bonding between perfectly matched λ-DNA molecules with lengths over 48,000 bp.

Alignment by flow also allowed for the spanning of disulfide-labeled λ-DNA molecules across the electrodes. A drop of DNA solution was centered over the electrode gap and then slowly moved across the gap using a light flow of nitrogen gas. However, this method proved to be much less reproducible and gave current values smaller in amplitude. The latter problem can most likely be attributed to the DNA strands not being focused into the gap. Instead, the molecules were probably evenly distributed over the electrodes with the majority not spanning the gap. This distribution was observed by AFM when alignment of short λ-DNA molecules by flow instead of an ac field was attempted (as will be described in Section 3.4.2).
**Figure 3-3.** AFM images of a device after an ac field (10^6 V/m, 1 MHz) had been used for the alignment of disulfide-labeled λ-DNA molecules across an 8 µm Au electrode gap. The device was fabricated on mica and imaged using non-contact mode scanning. (a) Larger view of electrode gap, depicting multiple bundles spanning the gap. (b) and (c) Closer views of bundles, each containing a large number of individual DNA strands.
3.4. Results with Disulfide-Labeled Short $\lambda$-DNA (7460 bp)

3.4.1. Characterization of Digestion Product

To characterize the result of the enzymatic digestion of $\lambda$-DNA with $Bp$/I, slab gel electrophoresis was utilized. Figure 3-4 displays a gel containing a 1 kb DNA ladder for sizing and the restriction enzyme cut $\lambda$-DNA. By comparing the cut molecules to the DNA ladder, their size could be estimated. It was found that the digestion yielded two lengths: a fragment with a size of $\sim$7500 (the expected length is 7460 bp or $\sim$2.5 $\mu$m) and a second fragment of $\sim$40,000 bp (the remainder of the $\lambda$-DNA). The Hoechst assay was also utilized to confirm that the disulfide labeling of these molecules was successful.

3.4.2. Alignment Across 1.2 $\mu$m Gaps

The 1.2 $\mu$m electrode gaps used for alignment and measurement of short $\lambda$-DNA were fabricated on SiO$_2$ substrates with substantially less roughness (approximately $\pm$ 0.2 nm), which allowed for some AFM imaging. After systematically testing various field strengths ($8 \times 10^4$ to $2 \times 10^6$ V/m) and frequencies (400 kHz to 8 MHz) reproducible alignment of a large quantity of these shorter strands by dielectrophoresis was still not achieved. The most promising alignments were achieved by ramping both the field and its frequency after adding a droplet of DNA, from $8 \times 10^4$ V/m to $5 \times 10^5$ V/m and 6 MHz to 1 MHz. Figure 3-5 shows a DNA strand spanning the gap after use of this technique. This strand is quite difficult to image amongst the other strands of DNA in the electrode gap that are not aligned. Most research completed thus far on the use of dielectrophoresis for the orientation and stretching of DNA have examined frequency and field parameters.
Figure 3-4. Image of a gel containing a 1 kb DNA ladder in lane 1 (with sizes in bp as shown) and the product from the digestion of λ-DNA with BspI in lane 2. The gel contained 1% agarose in 1×TBE with ethidium bromide dye for visualization.
Figure 3-5. AFM image of a device with a 1.2 µm gap after alignment of short disulfide-labeled λ-DNA. Alignment attempted using a ramping up of the field (8×10⁴ V/m to 5×10⁵ V/m) and a ramping down of the frequency (6 MHz to 1 MHz), immediately after adding the DNA solution to the electrode gap. The arrows point to a single strand spanning diagonally across the gap.
necessary for long molecules (~16 µm). While it is known that smaller molecules are less polarizable and therefore, require larger dielectrophoretic forces for orientation a study has not been completed on the frequency dependence for the stretching of fragments <16µm. Interestingly, Germishuizen et al. demonstrated a non-linear relationship between frequency and λ-DNA stretching with maximum stretching occurring between 300 and 500 kHz and decreasing both below and above this range. It is possible that the field strengths used were not high enough to focus and stretch a large number of these relatively short molecules in the electrode gap and/or the optimal frequency/field combination was never achieved in the attempted alignments. This latter hypothesis is supported by the fact that the most promising results were obtained when a range of field strengths and frequencies were applied to the same sample.

Other methods of stretching were tried, but these also yielded only limited success. These included continuous flow of a dilute DNA solution (0.05 nM) through a teflon chamber, across the device, using a low-volume pump, both with and without the application of an ac field. Alignment was also attempted by moving single or multiple droplets of 5 nM DNA across a device, either parallel or perpendicular to the electrode geometry, with the device placed on a flat or angled surface. The rate of flow using this technique was varied by adjusting the amount of nitrogen gas applied to the droplet(s). The receding meniscus method, where DNA is aligned as a droplet dries, seemed to be the best of these alternative procedures for molecule stretching. Figure 3-6 shows an AFM image of a device after using this method. While molecules appear fully stretched
Figure 3-6. AFM image of the edge of the Au electrode and gap after alignment of short disulfide-labeled λ-DNA by a receding meniscus. White arrows point to some of the DNA strands and bundles present on this bottom edge.
i.e. \( \sim 2.5 \) \( \mu \)m in length, the strands are mostly on the edge of the electrodes and not across the gap. Like the full-length \( \lambda \)-DNA on the 8 \( \mu \)m gaps, this can be attributed to the inability of this method to focus the DNA. However, with the short \( \lambda \)-DNA even less success was obtained. This could be a result of the difference in electrode geometry between the two devices as shown in Figure 2-3 (b) and (c). The devices with 8 \( \mu \)m gaps had open electrodes while the 1.2 \( \mu \)m gaps were filled and therefore, had a much larger gold surface area available. It is likely that upon placing a droplet of the short \( \lambda \)-DNA solution on a device, the majority of these molecules were immobilized on the Au via their disulfide groups, without any opportunity for alignment across the gap.

In conclusion, the reproducible alignment and attachment of large quantities of disulfide-labeled \( \lambda \)-DNA (16 \( \mu \)m) was achieved by manipulation with ac electric fields. Confocal microscopy could be used to confirm the success of this method by establishing the relative quantity of these aligned molecules between the electrode gaps. Conditions necessary for the reproducible alignment of a large number of short \( \lambda \)-DNA molecules across reduced gap sizes still need to be determined. However, the information that can be gained from the AFM imaging of these electrode gaps after attempts at alignment optimization (such as the exact location of DNA strands, the extent of DNA stretching, and an accurate estimate of strand quantity) should allow for these conditions to be established.
Chapter 4. D1S80 Results

Doubly functionalized D1S80 samples were successfully amplified using the procedure described in Chapter 2. Electropherograms of the amplified samples with thiol end groups are shown in Figure 4-1. Samples that possessed two peaks were heterozygous (the alleles from each chromosome in the homologous pair were not identical), while samples that gave one peak were homozygous (the alleles from each chromosome in the homologous pair were identical). A homozygous sample was chosen for electrical conductivity measurements so that only one possibility would exist for the length of DNA strand that was measured. The fragment length of the D1S80 allele used was 561 bp or approximately 190 nm, sufficient to span the fabricated 120 or 180 nm electrode gaps.

4.1. Attachment Assays

Qualitative measurements of the attachment of both thiol-labeled and amine-labeled D1S80 fragments to gold surfaces were completed using confocal microscopy and the fluorescent intercalating dye, TOTO-1. Both functional groups were found to allow the DNA strands to remain on the substrate, even after vigorous rinsing. Therefore, two different methods to functionalize DNA and provide immobilization were successfully demonstrated. These results were in agreement with previous research on the adsorption of both thiols\textsuperscript{108} and amines\textsuperscript{122} to gold surfaces.

A more quantitative assessment of thiol-Au attachment was completed using capillary electrophoresis and LIF detection. Figure 4-2 displays electropherograms obtained prior to and after adding a gold strip to a solution containing both thiol-labeled
Figure 4-1. Electropherograms displaying the separation of PCR amplified, thiol-labeled D1S80 alleles and the D1S80 allelic ladder. (a) Homozygous sample. (b) Heterozygous sample. Samples were separated using 7.3% poly(dimethylacrylamide) in 100 mM TAPS buffer. Detection was accomplished using an Ar-ion laser and YO-PRO-1 fluorescent intercalating dye.
Figure 4-2. Electropherograms depicting a 561 bp, thiol-labeled D1S80 allele, before and after the addition of a gold strip. (a) Solution containing this allele. (b) Same solution as in (a), after the addition of a gold strip for 15 min. Samples were separated using an ABI 310 with POP-4 as the sieving matrix. LIF detection was accomplished by mixing the sample with TOTO-1 iodide and using an Ar-ion laser for excitation.
D1S80 fragments and TOTO-1 dye. By using this assay it was demonstrated that the thiol-labeled DNA strands were binding to the gold surface with close to 100% efficiency.

4.2. Alignment

Preliminary attempts to align DNA across electrode gaps were completed using D1S80 samples. At this point in the dissertation research, general parameters necessary for the alignment of thiol-labeled DNA using an electric field had not yet been established, i.e. alignment was not completed in a humidity chamber for 25 min with an ac field, followed by 25 min without a field. Instead, alignment of the 561 bp, thiol-labeled D1S80 alleles across electrode gaps was attempted by utilizing dc fields. Furthermore, instead of having the field applied to the electrodes prior to droplet addition, 10 µl of ~50 nM D1S80 in its PCR amplification buffer was added to the device and then a field of $1 \times 10^5$ V/m was applied. Following a 5 min alignment at ambient conditions, the device was rinsed with deionized water and measured.

4.3. $I-V$ Measurement

An initial measurement of thiol-labeled D1S80 fragments yielded a linear $I-V$ curve from which an electrical resistance of ~5 MΩ could be estimated. Upon adding a solution of the enzyme Deoxyribonuclease I (DNAse I) in its corresponding reaction buffer to the electrode gap and incubating the device at 37 °C for 45 min, the $I-V$ curve was reduced to its air gap values, i.e. the values measured between the bare electrodes before trapping DNA. DNAse I is an endonuclease that digests single and double stranded DNA. It catalyzes the hydrolytic cleavage of phosphodiester linkages in the
DNA backbone in a statistically random fashion. However, even though the current values decreased with the addition of this enzyme, it is possible that the original values measured were due to a salt bridge that had formed between the gold electrodes. The small size of the gap and the absence of any filtration of the DNA sample prior to its application on the device, increased the likelihood of this. Furthermore, upon placing the additional solution (DNAse I and buffer) on the device, a salt bridge could have also disintegrated, resulting in the reduction of the current values observed. Unfortunately, the 120 and 180 nm device gap sizes were too small to assay for the presence or absence of DNA using confocal microscopy and a fluorescent intercalating dye, due to the limited resolution of the technique. Instead scanning electron microscopy (SEM) was used, however this technique could only confirm the integrity of the device, not determine the type of particles that were in the electrode gap. For example, utilizing SEM, it was found that these small gaps were easily destroyed by electrostatic discharge as shown in Figure 4-3.

Due to the inability to use a visualization technique to confirm the presence of D1S80 molecules between the electrodes and to help with the optimization of parameters necessary for the alignment of these molecules, a significantly longer molecule, λ-DNA, was examined in subsequent DNA investigations. This molecule, with a length of 16 µm when fully stretched, was capable of spanning much larger electrode gaps. Therefore, confocal microscopy could be used to visualize the presence and determine the relative quantity of these DNA strands.
Figure 4-3. SEM image of a device with two 180 nm gaps. Electrostatic discharge has caused the partial lift-off of both Au electrode gaps.
Chapter 5. \( I-V \) Measurements of Unlabeled \( \lambda \)-DNA and Repaired versus Nicked Disulfide-Labeled \( \lambda \)-DNA

5.1. Results of Unlabeled \( \lambda \)-DNA

As a starting point for \( \lambda \)-DNA research, initial \( I-V \) measurements were completed on unmodified molecules. These molecules were aligned between the gold electrodes using an ac field with a strength of \( 10^6 \) V/m and a frequency of 1 MHz for 25 min (see Section 3.3.2). Devices were also kept an additional 25 min in the hydration chamber after the field had been removed. Although this step was only necessary for Au-disulfide bond formation, most of the experimental parameters were kept the same so that the unmodified and disulfide-modified samples could be directly compared. However, because the \( \lambda \)-DNA was initially unmodified, the deionized water rinse that was part of the measurement protocol could not be used without also removing the DNA strands. Therefore, to ensure that it was DNA strands that were being measured and not salts or other artifacts, the \( \lambda \)-DNA solution was passed through a Microcon YM-100 filter. The filtrate from this, which contained the buffer salts that were small enough to pass through the pores, was then measured following the normal ac alignment procedure. It was found that this solution gave \( I-V \) values approximately equivalent to the air gap (open electrode measurement) of that device, i.e. a few picoamperes (pA). On the other hand, the retentate, containing the \( \lambda \)-DNA molecules did give a finite conduction as shown in Figure 5-1. However, these unmodified \( \lambda \)-DNA samples gave current values approximately one order of magnitude smaller in amplitude than the functionalized DNA
Figure 5-1. (a) Sketch of the unlabeled λ-DNA molecule. This molecule possesses overhanging, 12 base long arms which are ideal for modification. (b) Current-voltage characteristic measured at room temperature on unlabeled λ-DNA molecules on an 8 µm Au electrode gap. The I-V curve for the device without DNA (the air gap) is also shown.
samples that will be described next. This result demonstrates the importance of having good electrical contacts.

5.2. Results of Disulfide-labeled $\lambda$-DNA with Two Nicks and the Repaired Samples

$\lambda$-DNA molecules functionalized with disulfide groups at their 3′ ends were synthesized utilizing the overhanging regions of this molecule. The hybridization procedure of Braun et al. was used with minor adaptations as described in Section 2.2.49. Disulfide-labeled single strand sequences were hybridized to these complementary regions, but the phosphate backbones were left incomplete for some of the measurements, as illustrated in Figure 5-2.71 That is, gaps existed between the 5′ ends of the complementary single strands and the 3′ ends of the $\lambda$-DNA substrate. $I-V$ measurements made using this nicked-$\lambda$ were compared to an identical $\lambda$-DNA sample with the nicks repaired by enzymatic ligation (Figure 5-2). This figure also displays the air gap of the device as a zero line. For the nicked $\lambda$-DNA, the $I-V$ characteristic was pronouncedly non-linear, showing a conductivity gap up to $\pm 3$ V, beyond which sizable current flow occurred. Multiple voltage sweeps were completed on the same device, consistently yielding this curve shape. Sweeps were first performed in the negative to positive direction and then reversed. The overall shape of the data and even the fine structure present in the curve (at around 7 to 12 V), was found to be mirrored around the zero bias for up compared to down sweeps. This mirroring would be expected to occur if the source and drain electrodes hold interchangeable roles, as would be the case with a 48,502 bp random DNA sequence. Interestingly, the curves resulting from both the up
Figure 5-2. (a) Sketch of the disulfide-labeled λ-DNA molecule. The nicked DNA possesses two gaps in its phosphate backbones between the introduced 12 base oligonucleotides and the λ-DNA substrate. In the repaired DNA, the gaps between the 3’ and 5’ nucleotides were repaired using T4 DNA ligase. (b) Current-voltage characteristics measured at room temperature on disulfide-labeled λ-DNA molecules. The up sweep corresponds to data collected from a negative to a positive potential while the reverse is true for the down sweep.
and the down sweeps were asymmetric at high fields. Therefore, it appears that the gaps can also introduce a rectification effect.

Repaired λ-DNA showed a close-to-linear $I-V$ characteristic when the same potential sweeps were applied. At $\sim +13$ V, a very small plateau did appear in the $I-V$. This plateau was found to be present independent of sweep direction in this particular DNA sample and was always at a high positive bias. However, it was not present in every repaired DNA sample measured. At this point, a detailed understanding of the sources of this feature and the fine structure around 7-12 V bias in the nicked DNA samples, has not been attained.

An unambiguous comparison of the current values between the two curves is not possible. Even if the same concentration of DNA was added on different devices, the number of DNA strands captured and measured can vary due to uncertainties in the trapping procedure and the electrical contact formation. However, because reproducible $I-V$ curves could be obtained using this method, both for repeated measurements on the same DNA sample and measurements on different devices, these curve shapes can be compared and used to develop order of magnitude estimates of DNA conductivity. Assuming a monolayer of DNA was making contact with the electrodes, the dc conductivity of repaired λ-DNA was found to range between $7 \times 10^{-4}$ S cm$^{-1}$ and $1 \times 10^{-3}$ S cm$^{-1}$. These values are close to the literature average$^{40,41,43,46,47,51,52,56,123}$ and are well below the conductivities of undoped conductive polymers like polyacetylene ($\sim 10^{-2}$ S cm$^{-1}$).$^{124}$ Furthermore, by estimating that 25,000 DNA strands were present within this monolayer, a resistance of $\sim 10$ TΩ per DNA strand could be calculated.
On the other hand, the low-field (<3 V) conductivity of the nicked DNA was approximately a factor 20 lower than the repaired DNA. Therefore, it can be estimated that the two unrepaired gaps introduce an additional low field resistance of ~200 TΩ per DNA strand.

5.3. Parasitic Salt Contribution

There should have been only minimal amounts of salts present on devices during $I$-$V$ measurements of labeled samples because Microcon filtration was performed on the solution after the final hybridization or enzymatic step. Furthermore, since the DNA was attached via a Au-disulfide bond, a deionized water rinse could be used after alignment. However, to quantify parasitic conductance resulting from traces of enzymes and/or buffer salts, a baseline measurement was completed on a ten-fold excess solution of ligation buffer containing T4 DNA ligase. After using the normal alignment/attachment procedure, the droplet was dried on the device with a flow of nitrogen gas. The maximum value of the current measured, using a sweep from –20 to 20 V, was 1 nanoampere (nA). In contrast, devices with filtered disulfide-labeled DNA yielded several tens of nA after rinsing. Furthermore, Zhang et al. have shown that time-dependent hysteresis in the $I$-$V$ curves of DNA samples is an indication of residual salts on the device. In the current experiments, the up and down voltage sweeps closely mirrored each other and a sweep rate dependence was not observed. Therefore, by using the above described procedures for DNA measurement, parasitic conductance due to salt bridges was likely minimal.
5.4. Results of Disulfide-Labeled $\lambda$-DNA with Three and Nineteen Nicks and After Repair on the Device

The effect of three nicks (introduced using the second method for disulfide labeling in which the $\lambda$-DNA is bound to three complementary oligomers but not ligated, Section 2.3.2) and nineteen nicks (introduced using the enzyme, N.Bpu101, which cleaves the phosphate backbone of $\lambda$-DNA at nineteen recognition sequences) was also investigated. In agreement with the study completed on $\lambda$-DNA with two nicks, the conductivity of these damaged molecules was found to be reduced. Figure 5-3 shows the normalized $I-V$ characteristics of undamaged $\lambda$-DNA and $\lambda$-DNA with two, three, and nineteen nicks. As the number of nicks increased, the size of the conductivity gap lengthened, until after introducing nineteen nicks, the curve was only slightly above the air gap of the device. By using confocal microscopy and a fluorescent intercalating dye it was determined that DNA was still present in the electrode gaps when these lower $I-V$ values were obtained for nicked DNA samples. Furthermore, the $\lambda$-DNA sample with three nicks could be partially repaired after alignment and attachment on the device, using T4 DNA ligase (Figure 5-4). After treatment with this enzyme the sample’s $I-V$ characteristic became similar to the curve observed for disulfide-labeled $\lambda$-DNA with two nicks. It is likely that the linear characteristic of the sample was not totally restored because T4 DNA ligase efficiency was reduced on the device as compared to in a vial. In this situation, certain DNA strands may be blocked by other overlapping strands. This idea was supported by the lack of any improvement in the $I-V$ of $\lambda$-DNA possessing 19
Figure 5-3. (a) Sketches of the various structural forms of disulfide-labeled $\lambda$-DNA molecules that were measured. The $\lambda$-DNA with three nicks was synthesized by following the procedure for labeling the 5’ and 3’ ends of the same strand, however the three introduced oligonucleotides were left unligated to the template. The $\lambda$-DNA with nineteen nicks was created by using the endonuclease, N.$Bpu$101, to nick the phosphate backbone of this molecule at all of its recognition sites. (b) Current-voltage characteristics for disulfide-labeled $\lambda$-DNA molecules with various amounts of nicking. All data has been normalized to itself, except for DNA with 19 nicks which was normalized by using the largest current value obtained for the ligated DNA (0 nicks).
Figure 5-4. Current-voltage characteristics for disulfide-labeled λ-DNA with three nicks and after repairing the sample by adding T4 DNA ligase in its reaction buffer to the device for 6 h at ambient conditions.
nicks, after attempted repair on the device using T4 DNA ligase. Too many of the original 19 nicks probably remained, preventing a change in curve shape and current amplitude.

In conclusion, the different $I-V$ characteristics found for nicked and repaired DNA molecules suggest that DNA molecules can be modified to control their electronic properties. These studies also offer some insight into the mechanism of conduction in DNA. Although each nick was present in only one strand of the helix, the gapless complementary strand did not appear to shunt the current. This indicates that the complete double helical structure is involved in charge transport. Previous NMR studies have shown that nicking an oligonucleotide can introduce local distortions and destabilization of the double helix. Therefore, it is speculated that a nick in the phosphate backbone causes a loss in the base pair stacking, leading to poor overlap of the electron orbitals in that region. In this way, charge transport through the $\pi$-stack could be affected by a manipulation in the phosphate-sugar chain.
Chapter 6. \(I-V\) Measurements of Diversely Disulfide-Labeled \(\lambda\)-DNA and Disulfide-Labeled Short \(\lambda\)-DNA

6.1. Results of Two Disulfide Configurations

In an additional study the effect of disulfide end group position on the measured \(I-V\) characteristics was examined.\(^72\) Figure 6-1 illustrates the two types of disulfide configurations synthesized, with one label on the 3' end of each strand (type I) or with the disulfide groups on the 5' and 3' ends of the same strand, synthesized using a 25 base extension (type II). Because of this extension, type II DNA had an additional length of 13 base pairs. However, it is not expected that this short addition modifies charge transport because of the overall size of the \(\lambda\)-DNA molecule, \(~48,500\) bp. Therefore, a comparison between the \(I-V\) characteristics of type I and type II DNA can be made.

Figure 6-1 depicts representative \(I-V\) curves measured on the samples under ambient conditions. Multiple measurements are shown for type II DNA, including up and down voltage sweeps on the same sample and also \(I-V\) characteristics for samples on two different electrode devices (devices A and B), to demonstrate reproducibility. No apparent difference was observed between the two disulfide configurations and both gave a close to linear \(I-V\) curve. Assuming a monolayer of molecules was in electrical contact with the Au electrodes, a dc conductivity of \(7 \times 10^{-4} \text{ S cm}^{-1}\) to \(1 \times 10^{-3} \text{ S cm}^{-1}\) was estimated for either type of DNA. While it is likely that a large contact resistance was present in these measurements, curve shapes did change upon DNA manipulation as
Figure 6-1. (a) Sketches of the two types of disulfide-labeled λ-DNA configurations. For type I DNA, the disulfide groups were attached on opposite strands, while for type II DNA, the disulfide groups were attached on the same strand. λ1, λ2, λ3, and λ extension were oligonucleotides utilized in the synthesis. (b) Current-voltage characteristics of type I and type II DNA measured at room temperature.
demonstrated in the studies of nicked vs. repaired λ-DNA described in Chapter 5. This indicates that features resulting from the DNA strands could still be observed.

One would expect that if either disulfide configuration modified the charge transport, the effect would be apparent in the $I-V$ curve, either as a non-linearity or as an added resistance. However, since neither effect was observed, it can be concluded that the location of the two disulfide end groups, either on the 5′ and 3′ end of the same strand or on the 3′ ends of each strand, does not alter the overall charge transport properties of the molecule. Furthermore, these studies demonstrated that charge transport does not favor a single DNA strand. Instead, the double helical structure of the molecule is utilized, regardless of the contact configuration.

6.2. Results of Short λ-DNA

To determine the effect of both strand length and the effect of nicking a shorter length of DNA, the $I-V$ characteristic of a λ-DNA fragment with a length of 7460 bp was examined. One of the $I-V$ curves obtained for short λ-DNA fragments is shown in Figure 6-2. The dc conductivity for this sample was estimated at $8 \times 10^{-4}$ S cm$^{-1}$. However, this result cannot be directly compared to the conductivity estimate obtained for full-length λ-DNA because of difficulties in the alignment of the short λ-DNA fragments and therefore, differences in the quantity of strands spanning the electrode gaps on these devices. For this reason, nicking of the short λ-DNA was not explored. Further attempts are required to optimize the ac alignment of these molecules or to maximize their alignment by flow. The latter method can probably be accomplished with greater success
Figure 6-2. Current-voltage characteristic of short disulfide-labeled λ-DNA (7460 bp) measured at room temperature.
by utilizing a different device geometry. For example, open Au electrodes (as described in Sections 2.9.2 and 3.4.2), running parallel over a greater length would allow for more molecules to be adsorbed at the edges of the electrodes.
Chapter 7. \textit{I-V} Measurements of Single Stranded versus Double Stranded \(\lambda\)-DNA

Initial theoretical research has demonstrated that the native double helical structure is important, at least to some extent, in charge transport.\textsuperscript{24,126,127} This work, along with experimental studies on nicked vs. repaired \(\lambda\)-DNA and \(\lambda\)-DNA with disulfide end groups on the same strand vs. opposite strands completed in this dissertation research offer some insight into the mechanism of charge transport in DNA.\textsuperscript{71,72} However, by making direct measurements and comparisons of charge transport in single stranded \(\lambda\)-DNA versus double stranded \(\lambda\)-DNA it was possible to gain a better understanding of the relationship between structure and the molecule’s electronic properties.\textsuperscript{73}

7.1. Structural Difference Between Single Stranded and Double Stranded DNA

It is known that heating a solution of DNA can cause the double helix to separate and denature. By measuring several physical parameters as a function of increasing temperature, researchers have found that the DNA also undergoes a major structural change during this denaturation process.\textsuperscript{128} Both the optical activity and intrinsic viscosity are reduced with heating. These results indicate that the DNA molecules become less helical and more flexible. Furthermore, direct mechanical measurement of the force versus extension of single stranded DNA indicates a persistence length much shorter than the canonical double stranded DNA length.\textsuperscript{129} DNA persistence length is defined as the average extension along the \(z\)-axis of an indefinite length polymer.\textsuperscript{130} In other words, the persistence length is the length of the chain that behaves as a rigid rod.
DNA strands that are much longer than their persistence length form random coil-like structures while DNA strands that are equivalent to or shorter than their persistence length, are essentially linear. A persistence length of approximately 10 Å has been estimated for ssDNA as compared to the 500 Å or 150 base pairs for dsDNA, when the molecules are in 10 mM Na⁺ (these lengths are dependent on the ionic strength of the solution). Therefore, ssDNA is typically treated as a highly flexible polymer, lacking the helical structure that is formed when two DNA strands base pair.

### 7.2. Results of Single Stranded versus Double Stranded DNA

$I-V$ characteristics of both disulfide-labeled double stranded λ-DNA and disulfide-labeled single stranded λ-DNA were measured. By developing a labeling procedure to prepare λ-DNA with disulfide groups on the 3′ and 5′ end of the same strand, a method for measuring ssλ-DNA conductivity could also be developed. In these experiments, the unlabeled complementary strands were removed and just the 3′, 5′-labeled ssλ-DNA strands were immobilized across the gold electrodes. The ssλ-DNA was formed from dsλ-DNA using two different methods: a thermal/chemical denaturation and an enzymatic digestion utilizing lambda exonuclease. The ssλ-DNA and dsλ-DNA were then aligned between electrodes spaced 8 μm apart using an ac electric field of $10^6$ V/m. After alignment, the devices were rinsed with deionized water to remove unbound DNA, enzyme, buffer solution, and/or denaturant. The resulting $I-V$ characteristics of both the double stranded and single stranded samples were nearly linear when measured at room temperature. However, the ssλ-DNA samples consistently gave conductivity
values about two orders of magnitude smaller in amplitude as shown in Figure 7-1.

Assuming a monolayer of DNA is in contact with the Au electrodes, dc conductivity can be estimated at $7 \times 10^{-4} \text{ S cm}^{-1}$ for this dsλ-DNA compared to $7 \times 10^{-6} \text{ S cm}^{-1}$ for ssλ-DNA. By using confocal microscopy and TOTO-1 dye it could be confirmed that DNA was still present in the electrode gaps when these values were obtained for the ssλ-DNA samples.

In addition, consistently low current values were obtained for ssλ-DNA, whether the sample was made single stranded in a vial before alignment on the device, or if it was made in situ using enzymatic digestion or denaturation following alignment of the dsλ-DNA. By using the in situ method, it could be ensured that the ssλ-DNA molecules were aligned and attached to the electrodes in the same manner as the dsλ-DNA. Furthermore, since similar results were obtained for both methods of single strand formation (λ-exonuclease or formamide) as depicted in Figure 7-2, the difference in the I-V characteristics between dsλ-DNA and ssλ-DNA must be a result of the presence or absence of the double helical structure, and not a consequence of the procedure.

Therefore, in agreement with previous studies, the dominant factor influencing charge transport in DNA appears to be the molecule’s stacked base pairs with their overlapping π-orbitals, and not the phosphate backbone with its corresponding hydrated counterions.

7.3. Temperature Dependence

While determination of the absolute values of conductivity for different types of DNA is important, characteristic temperature dependences of this conductivity can also
Figure 7-1. Current-voltage characteristics for disulfide-labeled double stranded λ-DNA and disulfide-labeled single stranded λ-DNA (produced using λ-exonuclease or by heat denaturation in formamide). Inset shows the same $I$-$V$ curves for single stranded λ-DNA along with the air gap of the device, with the current axis appropriately scaled.
Figure 7-2. Current-voltage characteristics for single stranded disulfide-labeled $\lambda$-DNA formed using either an enzymatic reaction or by thermal/chemical denaturation. As indicated, some samples were made single stranded on a device after alignment of a double stranded sample, as opposed to being prepared in a vial prior to alignment and attachment.
help to determine the mechanism(s) of charge transport through the $\pi$-stack. For this reason, preliminary measurements on liquid nitrogen-cooled double stranded $\lambda$-DNA molecules were completed. The molecule’s resistance was found to increase as it was cooled. A strong temperature dependence is typical of a conductivity determined by a temperature driven process.$^{43,47,59}$ Therefore, this initial result indicates that thermally activated hopping may be a mechanism for the conduction process in $\lambda$-DNA molecules.
Chapter 8. *I-V* Measurements of DNA Fibers

By performing electrical conductivity measurements on macroscopic DNA fibers containing bundles of DNA strands, one can gain a better understanding of the contribution of other conduction paths besides the $\pi$-stack. Due to the lack of DNA alignment on the devices, it is likely that the majority of the DNA strands in the fiber are not spanning the gap (i.e. one end on one electrode and the other end on the second electrode). Therefore, these experiments instead provide an estimate of the contribution due to interstrand transport and ionic/water conduction along the phosphate backbone.

8.1. Two-Electrode Device

In Figure 8-1 optical images of two different calf thymus DNA fibers are displayed, demonstrating the variation in width that occurred when the procedure described in Section 2.13 was utilized. Below these is an image of a 15 $\mu$m wide fiber with both ends connected to indium electrical contacts for *I-V* measurements. Very low current values (<3 pA for ± 20 V sweeps) were observed for these and other DNA fibers at ambient conditions (72 °F, 22% RH), whether they were contacted to gold pads separated by 8 $\mu$m gaps by using mechanical pressure, or directly contacted by indium as shown, with >200 $\mu$m gaps. Higher current values (>100 nA for ± 20 V sweeps) were only obtained if the DNA strands were completely hydrated (kept in a humidity chamber at >95% RH for more than 6 h), Figure 8-2. But following this hydration, the *I-V* characteristics were significantly different than what was typically observed for disulfide-
Figure 8-1. (a) and (b) Optical images of two different calf thymus fibers, one with a width of ~22 µm and the other with a width of ~80 µm, demonstrating the variety in sizes achieved using the synthesis method described in Section 2.13. (c) A 15 µm wide fiber connected by indium contacts for its $I-V$ measurement. The spacing of this gap was 230 µm.
Figure 8-2. Current-voltage characteristics for a 22 µm fiber spanning an 8 µm Au electrode gap. Fiber contacted to electrodes by mechanical pressing via a compliant layer. After measurement at ambient conditions the fiber was placed in a humidity chamber for 6 h and re-measured. The up and down $I-V$ sweeps demonstrate the fibers hysteretic behavior after hydration.
labeled dsλ-DNA. The curves were very hysteretic and the current values dropped drastically after the fiber was removed from the chamber and began to dry. Within 10 min, the values had typically dropped by over three orders of magnitude and were again near the air gaps of the devices. The current values measured for disulfide-labeled dsλ-DNA samples only drop slightly after drying the devices by pumping under vacuum.

8.2. Four-Electrode Device

Measurements of calf thymus DNA fibers that were manually aligned across four-electrode devices, Figure 8-3, were also conducted. The gold electrodes on these devices were separated by 15 µm with a diagonal spacing of 21 µm. Fibers were held in place with a compliant layer and mechanically pressed to contact all four electrodes. This electrode geometry was designed to allow for direct comparison of charge transport across the length of the fiber versus transport across the width of that same fiber. While the current values measured across the width of these fibers were consistently low (<5 pA for potential sweeps of ±20V) at ambient conditions, some fibers did yield higher currents (1 to 4 nA for potential sweeps of ±20V) and close to linear I-V characteristics when measurements were collected across their length. After drying these fibers under vacuum for 1 h, the current values measured across their length using identical potential sweeps, did decrease but not to the same extent as the fibers measured using the alternative electrode geometries mentioned previously. The values were still almost an order of magnitude above the air gap, i.e. ~100 pA, and steadily increased with time as the fiber remained at ambient conditions and water was absorbed.
Figure 8-3. (a) Optical image of a 50 µm calf thymus fiber spanning the diagonal of a four Au pad geometry. The pattern was created using tungsten wires with a 15 µm diameter. The striations in the image are due to the compliant layer which was placed on top of the fiber so that it could mechanically contact all four electrodes. (b) Current-voltage characteristics obtained for this fiber when transport was measured across its length (by contacting Au 1 and Au 3) or across its width (by contacting Au 2 and Au 4) under ambient conditions or after vacuum pumping on the aligned fiber for 1 h.
Higher current values were obtained on the four pad Au electrode geometry when compared to the other geometries used for fiber measurements. This result was most likely caused by the difference in contact area. Contact resistance is inversely proportional to contact area and therefore, as contact area increases, the resistance should decrease.\textsuperscript{133} The 8 µm device geometry utilized thin gold electrodes with a width of 10 µm as shown in Figure 2-3 (b). By using this width and an average fiber diameter of 50 µm, the total contact area on these devices (including both electrodes) can be estimated at 1000 µm\(^2\). On the other hand, when the fiber was measured using the large Au square pads shown in Figure 8-3, a much greater contact area existed. The length of the fiber that was contacted to the gold via the compliant layer was \(~3500\) µm and by using an average fiber diameter of 50 µm, this yielded a contact area estimate of 175,000 µm\(^2\). Therefore, there was a difference of over 2 orders of magnitude for the contact areas of the two electrode geometries.

A difference in contact area also prevented conclusions to be reached regarding transport across the fiber width versus along its length. The use of a macroscopic compliant layer to contact the fiber to the four electrode pads led to a larger contact area when measurements were completed across the bundle’s length, as shown in Figure 8-3.

The approximate number of DNA strands in these fiber bundles can be estimated at 20,000-40,000, depending on the fiber width. This value is quite close to the monolayer quantity (25,000) that could be estimated for the disulfide-labeled \(\lambda\)-DNA spanning an 8 µm gap. However, comparisons between these functionalized DNA
strands with their chemical contacts to Au and the unlabeled calf thymus DNA strands with their mechanical contacts to Au may not be valid. It is more suitable to compare measurements of these fibers on 8 µm Au electrode gaps to the $I-V$ curves obtained for unlabeled λ-DNA, also spanning 8 µm electrode gaps. Even though the fiber measurements possess a larger contact area, their conductivity values were consistently over 3 orders of magnitude smaller in amplitude.

Overall, results on the calf thymus DNA fibers demonstrate that conduction due to pathways not involving the overlapping electron orbitals of the π-stack are minimal, unless the DNA fibers are extremely hydrated or their contact area is greatly increased. This result is in agreement with the $I-V$ measurements completed on nicked and single stranded, disulfide-labeled λ-DNA.
Chapter 9. Effect of Cations on DNA Structure and \( I-V \) Measurement

The interactions between cations and nucleic acids has attracted considerable interest because of the biological role that these ions play in the structure and function of DNA.\textsuperscript{134-136} It is known that some cations stabilize the double helix, while others or the same ions at higher concentrations can have deleterious effects on DNA and its biological function. Furthermore, certain transition metals can covalently bind to DNA bases, increasing the potential for these molecules to be used as molecular wires in nanoscale electronics.\textsuperscript{48,97,137,138}

9.1. DNA Counterions

In the past it was thought that interactions between the DNA phosphate backbone and cations could be represented as two rather extreme views.\textsuperscript{134,139} That is, that cations could bind tightly to the DNA after becoming partially dehydrated or that they could remain fully hydrated and be delocalized along the length of the polymer, free from direct contact. However, over the past few years, evidence from NMR spectroscopy, X-ray crystallography, and molecular dynamics simulations has suggested that the nature of nucleic acid-cation interactions actually lies between these two extremes.\textsuperscript{139} Whatever the case, it is likely that these counterions have some role in DNA conduction, either by influencing the structure of DNA which can then effect the overlapping electron orbitals or by providing an additional conduction path along this biomolecule.\textsuperscript{134} Therefore, changing the identity of these cations should influence charge transport. For this reason, determining the effect of counterion identity on the resulting disulfide-labeled \( \lambda \)-DNA \( I-V \) measurements is of interest.
In physiological systems, the cations that normally interact with nucleic acids are \( \text{Na}^+, \text{K}^+, \text{Ca}^{2+}, \text{and Mg}^{2+} \).\textsuperscript{139} After the enzymatic reactions involved in synthesizing the disulfide-labeled \( \lambda \)-DNA, the counterions that are present on the backbones of these molecules are primarily \( \text{H}^+ \) and \( \text{Mg}^{2+} \), due to the various reaction buffers used. To replace these cations, a very aggressive ion-exchange method was initially used where the DNA strands were directly exposed to relatively high concentrations (100 mM) of the desired cations in a buffer at pH 7. However, after repeatedly obtaining low current values for these ion-exchanged samples (barely above the air gaps of the devices) some of the used devices were examined by confocal microscopy with TOTO-1 intercalating dye. From the fluorescence intensity present on these devices, it could be determined that DNA strands were not spanning the electrode gaps to any appreciable extent. To establish the cause of this lack of alignment, AFM was used to image ion-exchanged DNA samples on mica surfaces.

9.1.1. Atomic Force Microscopy Characterization After Aggressive Ion Exchange

As mentioned in the experimental methods, MgCl\(_2\) was not added to these sample solutions prior to their deposition on the mica substrates used for imaging. Instead, MgCl\(_2\) was added directly to the substrate so that these strands would not be exposed to any additional cations in solution, which could influence their structures.

When a drop of B-form, disulfide-labeled \( \lambda \)-DNA solution is added to a Mg-coated mica surface, the DNA molecules spontaneously assemble into an evenly spread network over a large area (>50×50 µm) as shown in Figure 9-1. AFM images of the Li\(^+\)
Figure 9-1. AFM image of B-form disulfide-labeled λ-DNA adsorbed on a Mg$^{2+}$ coated mica surface.
ion-exchanged disulfide-labeled λ-DNA samples instead revealed significant amounts of DNA clumping as depicted in Figure 9-2. Based on the counterion condensation theory that Manning developed, condensation of DNA can become thermodynamically favorable when solvent-DNA interactions become less favorable than DNA-DNA interactions. It appears that this condition was achieved when this method of counterion exchange was used, i.e. enough of the negative DNA phosphate charges were neutralized that interstrand aggregation and intrastrand condensation were induced. This was likely the reason that the ac field that was normally used for disulfide-labeled λ-DNA alignment was not stretching and aligning these particular samples.

9.1.2. Atomic Force Microscopy Characterization After Drop Dialysis

By using the less aggressive drop dialysis procedure described in Section 2.14 we found that both the resulting Li⁺ and Na⁺ ion-exchanged disulfide-labeled λ-DNA strands were not aggregated and individual strands did not appear to be condensed as shown in Figure 9-3. Therefore, it appears that this method can be used for ion-exchange without inducing a harsh change in the DNA’s structure that would prevent its alignment across the electrode gaps utilized. I-V measurements of these samples have not yet been completed.

9.2. Intercalating Divalent Cations

While some metal ions interact with the phosphate groups of the DNA backbone non-covalently, other ions also have large affinities for DNA bases. It is well known that divalent metal cations such as Zn²⁺, Co²⁺, or Ni²⁺ can replace the imino protons of
Figure 9-2. AFM images of disulfide-labeled λ-DNA with Li\(^+\) counterions on two different areas of a Mg\(^{2+}\) coated mica surface. This sample was formed using the aggressive ion exchange method.
Figure 9-3. AFM image of disulfide-labeled λ-DNA with Li\(^+\) counterions on a Mg\(^{2+}\) coated mica surface. This sample was formed using the drop dialysis ion exchange method.
DNA base pairs at pH conditions above 8, forming novel DNA-metal ion complexes termed M-DNA as shown in Figure 9-4. The proposed M-DNA structure is based upon NMR and circular dichroism results, and it is presumed that metal ion binding sites on the DNA backbone must be nearly saturated before this intercalation can occur. Preliminary studies have indicated that this chemistry can be utilized to enhance the biomolecule’s electronic properties. For example, Rakitin et al. directly measured the electrical properties of Zn-DNA and found that it exhibited metal-like conduction. These researchers theorize that the insertion of the metal ions in each base pair, regardless of sequence, could lead to more efficient transport if multistep hopping is the dominant mechanism. In other words, every metallated base pair would be able to participate in hole transport rather than just the randomly placed guanine residues in the B-DNA form.

**9.2.1. I-V Measurements**

In agreement with the results of Rakitin and colleagues, changes were observed in the I-V characteristics of DNA samples upon metallation. Figure 9-5 shows an I-V curve obtained for disulfide-labeled λ-DNA with two nicks and then after intercalation of Ni$^{2+}$ ions into this same DNA sample while it was on the device. The curve was found to become more linear without any observable gap after metallation. A similar improvement in conductivity was also observed upon metellation of unlabeled λ-DNA on poly-L-lysine. After exchanging the imino protons with Ni$^{2+}$, the conductivity of this sample was increased by a factor of 5 while the curve shape remained linear. Although
Figure 9-4. The proposed base-pairing scheme for M-DNA in which the imino protons of T and G are replaced by a Zn$^{2+}$ ion. Adapted from Reference 47.
Figure 9-5. Current-voltage characteristics obtained for disulfide-labeled λ-DNA with two nicks in its phosphate backbones and after that sample’s imino protons have been replaced with Ni$^{2+}$. 
some measurements with appreciable current values were obtained for M-DNA, these results were achieved when unmetallated DNA was first stretched and attached across the electrode gaps, and then that same sample was metallated on the device. When attempts were made to align DNA that was already in the M-DNA form on the device, utilizing the parameters that had been optimized for the B-form disulfide-labeled λ-DNA, significant current values were not measured. By utilizing confocal microscopy and Oligreen dye, it was determined that these M-DNA samples consistently had less fluorescence intensity within the electrode gaps as compared to the B-DNA samples. A search of the literature revealed that a consensus has not yet been reached on the structural effects of these transition metals binding to double stranded DNA. Furthermore, to the author’s knowledge, a study of the structural effects of divalent cations binding to single stranded DNA had not yet been completed. Therefore, to characterize structural changes that were occurring in the M-DNA samples, which could prevent reproducible alignment and attachment across the devices, capillary electrophoresis along with fluorimetry and atomic force microscopy were utilized. Information gained from these studies, including the size and flexibility of the M-DNA molecules, can help determine the electrode gap length and alignment parameters necessary to optimize $I-V$ measurements of these molecules.

9.2.2. Fluorimetry Results

Unlike B-DNA, TOTO-1 iodide fluorescent intercalating dye cannot bind to M-DNA, most likely due to charge repulsion. Therefore, by measuring fluorescence intensity, one can monitor the extent of M-DNA formation, i.e. the approximate
concentration of divalent cations bound to the oligonucleotides. This assay can also be
used to measure the effect of pH on divalent cation binding, the length of time necessary
for binding, and the stability of the nucleobase-cation bond once formed.

Utilizing this assay it was discovered that 3.0 mM concentrations of Fe\(^{2+}\), Zn\(^{2+}\), Co\(^{2+}\),
and Ni\(^{2+}\) at pH 8.3 reduced the fluorescence of \(\lambda\)-DNA with TOTO-1 dye to 91\%, 70\%,
6.2\%, and 2.9\% of the expected intensities, respectively. As will be discussed later, after
finding no effect on the resulting CE of DNA exposed to 3.0 mM Zn\(^{2+}\), this fluorescence
assay was performed at higher concentrations. By increasing the concentration of Zn to
20 mM and 100 mM, the fluorescence intensity was further reduced to 59\% and 41\%,
respectively.

Fluorescence measurements of \(\lambda\)-DNA with TOTO-1 in 3.0 mM Ni, 10 mM Tris-
HCl buffer at pH 8.3 versus pH 7 revealed a difference in intensities between the two pH
levels, indicative of the relationship between pH and metal incorporation in the DNA
helix (Figure 9-6). Furthermore, by monitoring TOTO-1/\(\lambda\)-DNA fluorescence over time
in the presence of 3.0 mM Ni at pH 8.3, it was found that after approximately 20 min the
fluorescence had reached its lowest value, just above the fluorescence intensity of the
blank (3.0 mM Ni in Tris-HCl buffer with TOTO-1 but no DNA). Once formed, M-DNA
samples appeared to be stable at least up to two weeks, giving an approximately constant,
low fluorescence intensity.

9.2.3. Capillary Electrophoresis Results

Many labs are now using CE as a precise method for DNA typing of bloodstains
and other body fluids from crime scenes. Such samples may occasionally come in
Figure 9-6. Representative results for the fluorescence assay utilized to monitor M-DNA formation. Samples consisted of 0.2 µg of λ-DNA with 150 µL of 3.0 mM NiCl₂ in 10 mM Tris, at pH 8.3 and pH 7 or with 150 µL 10 mM Tris, pH 8.3. After mixing the DNA with the buffer, the solution was allowed to interact for 1 h and then 0.2 µL of 0.25 mM TOTO-1 dye was added to each and the fluorescence was measured.
contact with transition metal cations. Therefore, besides gaining structural information about M-DNA, results on the ABI 310 Genetic Analyzer also illustrate the negative role that these divalent cations can have on DNA genotyping and demonstrate solutions to reverse this effect. Current protocols for DNA sizing using CE require that the analyses be performed on ssDNA samples to increase resolution. Therefore, dsDNA samples are placed in a chemical denaturant, formamide, and heated at 95 ºC to break hydrogen bonding. It was found that commingling of dsDNA samples with certain divalent transition metal cations in a typical buffer (pH > 8) can cause the peaks in the CE runs of the ssDNA samples to be poorly resolved, possess low fluorescence intensities, and have altered electrophoretic mobilities. The extent of these negative results was dependent on factors such as the identity of the divalent cation, the concentration of that cation, and the length and sequence of the DNA fragment. Conversely, DNA samples at pH 7 and those with cations incapable of causing an M-DNA conversion, such as Mg\(^{2+}\) and Fe\(^{3+}\), gave sharp, intense peaks with their expected mobilities. These results demonstrated that the CE effects were not due to ionic interactions between cations and the DNA phosphate backbone but instead were the result of covalent binding of metal ions to the DNA bases.

9.2.3.1. Effect of pH and Length of Time in Formamide for DNA Exposed to Nickel

Unlike fluorimetry studies, CE runs were completed under denaturing conditions. Therefore, in this section the results of interactions of the divalent cations with the DNA samples will be described as being due to the covalent binding of the metals to the DNA bases rather than an intercalation effect. When dye-labeled M-DNA samples were analyzed using CE and LIF detection, Ni, Co, and Zn-DNA gave results that were
significantly different than their pH 7 counterparts. Furthermore, each of the three divalent cations appear to interact with the DNA samples in a slightly different manner. The DNA samples that were examined included the fluorescently labeled allelic ladder, AMPFISTR Profiler Plus, which consists of the most common alleles present in 9 short tandem repeat (STR) locations or loci. These 9 STR loci are a subset of the 13 STRs used in the Federal Bureau of Investigation’s nationwide DNA database.\textsuperscript{147} Also used were GeneScan-500, an internal size standard containing 16 DNA fragments labeled with the red fluorescent dye ROX, and Y-PLEX 6, a reference ladder, which contains the amplified alleles for 6 Y-chromosomal specific STR loci.\textsuperscript{148} In addition, the fluorescein-labeled HUM-TH01 allele 9.3, an STR marker from the human tyrosine hydroxylase gene, was utilized for some CE experiments.\textsuperscript{149}

As can be seen in Figure 9-7, TH01, a 198 base pair fragment, and other DNA fragments exposed to Ni cations at pH 7 gave sharp, intense peaks. This result is expected for fragments having a homogeneous molecular mass, charge, and/or structure. On the other hand, the M-DNA samples and the ROX dye-labeled internal standards exposed to these samples, gave broad peaks or peaks with shoulders. This result indicates either a range of molecular masses, charges, and/or structures for the affected fragments or the adsorption of the sample to the capillary wall or sieving matrix. However, the idea that peak broadening is caused by adsorption can be eliminated because similar results were observed when the M-DNA was separated under native conditions with a fluorocarbon-coated capillary and a hydroxyethyl cellulose separation medium. As shown in the lower electropherogram of Figure 9-7, at this Ni\textsuperscript{2+}...
Figure 9-7. Electropherograms obtained on an ABI 310. 1 µL TH01 was added to 6 µL of 6.0 mM NiCl₂ in 10 mM Tris, pH 7 or pH 8.3. The samples were allowed to interact for 1 h and then 1 µL was added to ROX-labeled DNA/formamide.
concentration, the TH01 allele is so broadened, it has disappeared from the electropherogram. Other cations incapable of causing an M-DNA conversion, such as Mg$^{2+}$ and Fe$^{3+}$ were also mixed with the same DNA samples in Tris-HCl buffer at pH 8.3, and like the pH 7 samples, yielded normal peak shapes in the resulting electropherograms (Figure 9-8).

Examination of the metal-DNA complex, Ni-TH01 allele 9.3, as a function of time was particularly interesting. When 1 µL of the TH01 allele was added to 6 µl of 6.0 mM NiCl$_2$ in 10 mM Tris-HCl, pH 8.3, incubated for 1 h and then placed in ROX-labeled DNA/formamide, the TH01 allele was not visible and the ROX-labeled alleles (especially the primers and smaller fragments) were significantly broadened and reduced in intensity (Figure 9-7). However, by lowering the overall Ni concentration just slightly (1 µL of TH01 allele added to 6 µL of 3.0 mM NiCl$_2$), the TH01 allele was just visible as shown in Figure 9-9. The run taken immediately after adding this sample to ROX-labeled DNA/formamide revealed that the peak corresponding to this allele had a broad, faster migrating shoulder. But in a subsequent analysis of the same sample, the shoulder had switched positions, migrating at a slower velocity. By the third run this shoulder had started to disappear and the TH01 allele completely returned as a sharp peak without any shoulders by the 10$^{th}$ run, approximately 4.5 h later.

Figure 9-10 shows the results obtained for the Ni-Profiler Plus complex. While the resolution of the alleles exposed to Ni at pH 7 appear normal, their intensity is reduced when compared to the Profiler Plus that has not been exposed to any nickel ions.
Figure 9-8. Electropherograms obtained on an ABI 310. 1 µL TH01 was added to 6 µL of 6.0 mM MgCl₂ in 10 mM Tris, pH 7 or pH 8.3. The samples were allowed to interact for 1 h and then 1 µL was added to ROX-labeled DNA/formamide.
Figure 9-9. Electropherograms obtained on an ABI 310. 1 μL of TH01 was added to 6 μL of 3.0 mM NiCl₂ in 10 mM Tris, pH 8.3. The sample was allowed to interact for 1 h and then 1 μL was added to ROX-labeled DNA/formamide.
Figure 9-10. Electropherograms obtained on an ABI 310. 1 µL Profiler Plus was added to 6 µL of 10 mM Tris buffer or 10 µL of 10 mM Tris buffer with 6.0 mM NiCl₂, pH 7 or pH 8.3. The samples were allowed to interact for 1 h and then 1 µL of each was added to ROX-labeled DNA/formamide.
Using the fluorimeter with the fluorescein-labeled TH01 allele and the JOE, NED, and 5-FAM dye-labeled Profiler Plus allelic ladder, it was determined that the cations used did quench some dye fluorescence, the extent of which was dependent on the cation’s identity. \( \text{Co}^{2+} \) cations caused the most quenching, followed by \( \text{Ni}^{2+} \), \( \text{Zn}^{2+} \), and \( \text{Fe}^{2+} \). Therefore, the 50% reduction in intensity for the pH 7 sample in Figure 9-10 is probably largely due to this dye inhibition and not a result of the divalent cations binding to the DNA bases. Furthermore, since increased salt concentration in the CE sample solution has been shown to reduce injection efficiency, this phenomenon could also be contributing to the lower intensities.

Figure 9-11 shows the effect of the Ni cations on two alleles amplified using PCR. Initially, the two alleles were not visible but upon the next run (30 min later) they appeared, while the ROX-labeled DNA peak shapes deteriorated. After approximately 6 h, like the TH01 allele (Figure 9-9), both the PCR and ROX-labeled DNA peaks returned to their original, unaltered form. But at higher cation concentrations, (1 \( \mu \text{L} \) of amplified sample mixed with 6 \( \mu \text{L} \) or more of 3.0 mM NiCl\(_2\) solution) the affected alleles only partially returned or remained undetectable.

As mentioned previously, when the TH01 allele was exposed to concentrations above 3.0 mM NiCl\(_2\), the allele peak was no longer visible in the initial runs. It is suspected that this effect was due to the formation of a very broad peak that disappeared into the baseline of the electropherogram when this divalent cation was present at a high enough concentration. This was supported by the electropherograms depicted in Figure 9-12. This figure shows both the electropherogram obtained when an aliquot of 3.0 mM
Figure 9-11. Electropherograms obtained on an ABI 310. 1 μL of amplified sample was added to 0.5 μL of 10 mM Tris buffer with 3.0 mM NiCl₂, pH 7 or pH 8.3. The sample was allowed to interact for 1 h and then 1 μL was added to ROX-labeled DNA/formamide.
Figure 9-12. Electropherograms obtained on an ABI 310. 1 µL of either 3.0 mM or 10 mM NiCl₂ was added to 11.5 µL of ROX-labeled DNA/formamide.
Ni\textsuperscript{2+} was added to a solution of ROX-labeled DNA fragments in formamide and the electropherogram obtained when an aliquot of 10 mM Ni\textsuperscript{2+} was added to another solution of ROX-labeled DNA fragments in formamide. By increasing the amount of Ni ions added, the ROX peaks became even broader and further lowered in intensity. This is presumably due to more cations binding to the ROX-labeled DNA bases and therefore, a larger variety of stable structural forms being present for each fragment size.

**9.2.3.2. Reversibility of Negative Effects**

It was also possible to demonstrate that the broad, low intensity peaks characteristic of M-DNA samples were reversible. An aliquot of a solution containing the Ni-TH01 allele was added to formamide and measured on the 310 as shown in the upper CE in Figure 9-13. The pH of that same sample solution was then adjusted using a micro-pH meter to a level where metal-nucleobase binding could not occur and a second aliquot was then added to formamide. After this adjustment, the missing TH01 allele reappeared with the primer peaks also becoming sharper and more intense.

The effect of metal binding on the resulting electropherogram could also be reversed using EDTA to chelate the metal cations as shown in Figure 9-14. Note that while the peaks were sharp, the intensity of the alleles had been slightly lowered in the run where EDTA was added. This effect is likely due to less DNA being injected because of the higher salt concentration present in the vial.

Finally, M-DNA formation can typically be reversed simply by leaving the DNA fragments in formamide for 1-10 h. It was found that this length of time was dependent on the cation concentration present, i.e. DNA that was exposed to higher cation
Figure 9-13. Electropherograms obtained on an ABI 310. The pH of a Ni-TH01 sample was adjusted from 8.3 to 6.9 using HCl and a micro-pH electrode for measurement.
Figure 9-14. Electropherograms obtained on an ABI 310. 1 µL of TH01 was added to 6 µL of 6.0 mM NiCl₂ in 10 mM Tris, pH 8.3. The sample was allowed to interact for 1 h and then a 1 µL aliquot was added to 0.5 µL of 0.5 M EDTA in 10 mM Tris, pH 8.3 and a second 1 µL aliquot was added to 0.5 µL of 10 mM Tris, pH 8.3. After 2 h, an aliquot of each was added to ROX-labeled DNA/formamide for measurement.
concentrations required a longer time in formamide for an improvement in peak shape(s). Presumably, when the M-DNA sample is diluted in formamide, the divalent cations are often no longer in excess and are released from the affected DNA fragment. These released cations along with those in excess in the DNA sample solution then bind to the ROX-labeled DNA fragments resulting in a distortion of their peak shapes (as can be seen in Figure 9-11). This continues until an equilibrium is reached in formamide and the cations have redistributed between the solution and the DNA fragments. For example, with the Ni-TH01 sample shown in Figure 9-9, the return of peak shape and intensity of the TH01 allele began immediately and was complete after approximately 4.5 h. But it was not until about the 5th run (2 h later) that the shape of the ROX-labeled DNA peaks began improving and their peak shape did not fully return to normal until 9 h after the initial run. This reasoning is in agreement with the fact that at very high metal ion concentrations (when 1 µL of TH01 was added to 6 µL of 20 mM NiCl₂) the TH01 allele did not completely return to its normal intensity and shape, even after 24 h in formamide. The time dependency results were obtained whether different aliquots of an equilibrating sample were injected or the same sample was injected repeatedly. This fact, in addition to the difference in equilibrium times between the ROX-labeled DNA and the TH01 allele, demonstrates that the time effect was not an artifact of the electrokinetic injection.

9.2.3.3. Size and Sequence Dependence

Figure 9-15 demonstrates how the extent of peak broadening was found to be dependent on the DNA fragment’s size. For example, the smallest ROX-labeled DNA
**Figure 9-15.** Electropherograms obtained on an ABI 310. 1 µL of 3.0 mM NiCl₂ in 10 mM Tris, pH 8.3 was added to 11.5 µL of ROX-labeled DNA/formamide and the sample was measured over time.
fragments were the most negatively affected, i.e. broadened and lowered in fluorescence intensity to the greatest extent after Ni$^{2+}$ addition. This trend can also be seen clearly in the previous figures. Even though the 35 bp ROX fragment was the most drastically effected of the internal standard peaks, it was also the first to return to its normal shape and expected mobility with time in formamide. Its return was followed by the 50 bp ROX fragment, then the 75 bp fragment and so on.

In addition, it was determined that the effect of metal-DNA complexation on the resulting CE peak shape was somewhat sequence dependent. Figure 9-16 shows the CE run of Ni-Y-PLEX 6 ladder. The locus with the smallest fragments, DYS 393, and the locus with the largest fragments, DYS 385, were the most affected and were barely visible in the analyzed data while the resolution and fluorescence intensity of the alleles in the 5th locus, DYS 389 II, were the least affected. The Ni-Profiler Plus allelic ladder also showed similar locus-dependent results (Figure 9-10). The relationship between sequence and metal-DNA complexation has previously been examined by Dugid et al. and Wood et al.$^{135,152}$ Dugid and colleagues demonstrated that divalent cations preferentially bind to G/C regions while Wood’s group demonstrated that both poly(G/C) and poly(A/T) sequences formed M-DNA more readily than the corresponding DNAs with mixed sequences. Of the aforementioned loci, DYS 393, DYS 385, and DYS 389 II, it is DYS 389 II that has the largest G/C content although the peak shape of the alleles in this locus were the least affected of the three. However, upon examination of the sequences of the three loci it was found that the DYS 385 locus had multiple regions with poly(A/T) sequences, 5 bp or greater in length and the STR region contained
Figure 9-16. Electropherograms obtained on an ABI 310. 1 µL Y-PLEX-6 ladder was added to 6 µL of 6.0 mM NiCl₂ in 10 mM Tris, pH 7 or pH 8.3. The sample was allowed to interact for 1 h and then 1 µL was added to ROX-labeled DNA/formamide.
poly(GAAA). The DYS 393 locus also had a few regions of poly(A/T) sequences and had smaller fragment sizes, while DYS 389 II was longer and had a much more evenly distributed base content. Thus, in these experiments it appears that the poly(A/T) regions most strongly influence metal-DNA complexation, and not the overall G/C content.

9.2.3.4. M-DNA Mobility

As previously discussed, the binding of Ni$^{2+}$ to the DNA bases had an effect on DNA mobility. Figure 9-17 shows the fluctuation in the Ni-TH01 allele size from 195.2 to 197.0 bases as a function of the length of time the DNA remained in formamide. The lack of a consistent change in the size estimate with time is likely due to mobility shifts for the ROX-labeled DNA internal standard fragments as they too incorporate metal ions upon exposure to the Ni-TH01 allele. Conversely, the same experiment was performed using the trivalent ion, Fe$^{3+}$, and as expected, the TH01 allele size was quite stable with time in formamide, only fluctuating from 195.2 to 195.4 bases. This effect demonstrates the inability of this ion to covalently bind to the DNA bases.

Due to the fact that the size estimation can be affected both by metal ion incorporation in the target allele and also by metal ion incorporation in the ROX-labeled DNA standard, a fluorescent artifact peak (which had a higher mobility than the TH01 allele) was used as an unaffected size marker. Using this marker, it was clear that the Ni-TH01 allele initially had a higher mobility and then over the next few runs it approached its normal mobility with only minor fluctuations, Figure 9-18. This mobility trend also followed the allele’s shoulder position as shown in Figure 9-9. If one examined the change in the fluorescence intensity of this same allele with time there was a slightly
Figure 9-17. Ni-TH01 plot created using the data shown in Figure 9-9, along with the rest of the data set for that same sample. For the Fe exposed sample, 1 µL of TH01 was added to 6 µL of 15 mM FeCl₂ in 10 mM Tris, pH 8.3. Both Ni and Fe-TH01 were sized using ROX-labeled DNA positions from the initial CE run.
Figure 9-18. Both Ni-TH01 plots were created using data shown in Figure 9-9. Mobility was calculated by dividing the position of a fluorescent artifact peak by the position of the Ni-TH01 allele. The fluorescent artifact migrated at a higher mobility than the allele and therefore, had a lower position number. Only four data points are shown in the lower plot due to the allele intensity being off-scale by the fifth run.
different trend. Between the first run and the second run there was not much change, which was the opposite of what was observed in the mobility plot where the greatest change occurred between these two runs. However, between the second and fourth runs, the peak intensity changed drastically. The difference between the two plots in Figure 9-18 probably indicates that two phenomena are occurring over time due to the metal ions leaving the DNA bases. The initial mobility shift from high to low likely occurs due to a structural change. If it was just due to the molecules regaining their expected negative charge and lower mass, the shift should be in the opposite direction, i.e. from a low mobility to the higher, normal mobility. Instead this shift to higher mobility indicates that a condensed DNA structure is initially formed with the metal ions. As the metal ions leach out, the loss of this condensed structure takes place rapidly. Next, the various DNA conformers begin to convert into molecules with identical structures, a process that takes place at a slower rate. This increase in homogeneity by the third run is what results in the narrower peak and therefore, higher fluorescence intensity.

9.2.3.5. DNA Exposed to Zinc

Zn cations caused similar effects on the resulting electropherograms. However, a much larger concentration of Zn$^{2+}$ was necessary to cause these negative effects. After mixing various DNA samples with 3.0 mM and 20 mM ZnCl$_2$, the corresponding electropherograms appeared normal (besides a slight drop in allele intensity). Figure 9-19 shows a CE run of the TH01 allele after mixing it with 100 mM ZnCl$_2$ (at this and the 20 mM concentration, some of the Zn precipitates as the hydroxide after pH adjustment). Both the allele and the primer peaks were broadened and lowered in intensity in a manner
Figure 9-19. Electropherogram obtained on an ABI 310. 1 µL of TH01 was added to 6 µL of 100 mM ZnCl₂ in 10 mM Tris, pH 8.3. The sample was allowed to interact for 1 h and then 1 µL was added to ROX-labeled DNA/formamide. Second CE run shown.
similar to Ni-TH01. Initially, after adding the sample to the ROX-labeled DNA/formamide mixture the allele was not visible but by the second run (~30 min later), the broadened peak could be seen, but at a higher mobility than the normal TH01 without metal ions incorporated. Unlike Ni-TH01, the Zn-TH01 allele maintained its higher overall mobility and never regained its sharpness or intensity with time in the formamide solution. The absence of the return of these characteristics for the TH01 allele peak could be due to zinc cations, not bound to the DNA bases, remaining in solution when this higher concentration was used. This excess could prevent an M-DNA to B-DNA conversion even after dilution in formamide.

9.2.3.6. DNA Exposed to Cobalt

The Co-DNA also gave interesting results. While Ni and Zn produced negative results at 3.0 mM and 100 mM, respectively, a much lower concentration of CoCl₂ (0.05 mM) was sufficient to affect peak shape and intensity. At a 3.0 mM concentration no peaks (for the ROX-labeled fragments or the TH01 sample) were visible in the run. However, by lowering the initial Co concentration to 0.05 mM a very broad TH01 allele was just visible as shown in Figure 9-20. The effect of Co on this allele was similar to the effect of the other divalent cations. The Co-TH01 migrated at a reduced mobility and as a broad peak. However, unlike the Ni or Zn cations, the TH01 allele exposed to Co never exhibited a peak or shoulder with a higher mobility than expected. Instead it migrated at a reduced mobility until it regained its sharpness and intensity with time in formamide.
Figure 9-20. Electropherogram obtained on an ABI 310. 1 µL of TH01 was added to 6 µL of 0.05 mM CoCl$_2$ in 10 mM Tris, pH 8.3. The sample was allowed to interact for 1 h and then 1 µL was added to ROX-labeled DNA/formamide.
9.2.3.7. DNA Exposed to Iron

The Fe\(^{2+}\) cation did not appear to affect the peak shape like the aforementioned divalent cations but did affect the peak heights of the alleles in the CE runs. Fluorimetry assay results indicated that only a small amount, if any, Fe\(^{2+}\) can incorporate into the DNA helix. Furthermore, as previously mentioned it was determined that Fe\(^{2+}\) did cause some quenching of the dyes on labeled DNA. Together, the CE and fluorimetry results indicate that if Fe\(^{2+}\) is binding to the bases at all (and the lowered fluorescence intensity is not just due to dye quenching or reduced injection efficiency), this binding does not effect the structure of the DNA enough to cause a mobility shift and/or loss of resolution in the corresponding CE runs.

9.2.3.8. DNA Exposed to Other Contaminants

After initially investigating a series of divalent cations, DNA samples were exposed to an additional group of compounds to determine their effect, if any, on the resulting CE. Test compounds that caused no effect even at high concentrations were cetyltrimethylammonium bromide, sodium dodecyl sulfate, tetraethylammonium chloride hydroxide, Tween 20, formic acid, hematin, humic acid, indigo dye, and the following DNA dyes: oliggreen, picogreen, and Hoechst. Besides the divalent cations already mentioned, only spermine, poly-L-lysine, TOTO-1 dye, TOTO-3 dye, and TOPRO-3 dye caused abnormal CE runs for the DNA samples exposed to those materials. Experiments with spermine and poly-L-lysine involved mixing the DNA sample (TH01 allele or CTTv ladder) with the compound for 1 h before addition to ROX-labeled DNA/formamide. The spermine-exposed TH01 allele migrated with a much higher mobility but with a normal intensity and peak shape. Figure 9-21 shows that within the first hour of adding
Figure 9-21. Electropherograms obtained on an ABI 310. 1 µL of TH01 was added to 2 µL of 10 mM spermine in 10 mM Tris, pH 8.3. The sample was allowed to interact for 1 h and then 1 µL was added to ROX-labeled DNA/formamide. The chemical structure of spermine is shown in the 1st run.
this sample to the ROX-labeled DNA/formamide (2nd run), a second set of ROX-labeled peaks appeared at an increased mobility. It is likely that spermine, a polycationic species, which is known to stabilize duplex and triplex formation in DNA is causing the DNA samples to be double stranded even though they are in formamide.\textsuperscript{153} These results demonstrate that the negative CE effects observed in M-DNA samples are not the result of the formation of dsDNA since the spermine-affected DNA retained its peak sharpness.

Another polycationic species, poly-L-lysine, caused broadening of the DNA peaks and a lowering in their intensity, similar to the divalent cations as shown in Figure 9-22. This compound had the most negative effect on the peak shapes of the larger DNA fragments and also caused the introduction of additional peaks in the resulting electropherograms. For example, some of the large ROX-labeled DNA fragments had two extra fragment peaks, one migrating at a higher mobility than expected (but not fast enough to be caused by the DNA being double stranded) and one migrating at a lower mobility than expected.

The results that were most similar to the divalent cation affected-DNA were obtained for DNA samples with various intercalating dyes as shown in Figure 9-23. This figure shows a comparison of the results obtained when 0.1 µL of 0.05 mM CoCl\textsubscript{2} (pH 8.3) versus 0.1 µL of 0.05 mM TOTO-3 were introduced into 24 µL of ROX/formamide and then the solutions were heat denatured, snap-cooled, and run on an ABI 310. The CE pattern was quite similar between the two samples with the smaller fragments being least effected. Furthermore, both electropherograms display peak tailing for ROX fragments smaller than 200 bases and peak fronting for fragments larger than 200 bases. Of the
Figure 9-22. Electropherogram obtained on an ABI 310. 1 µL of CTTv allelic ladder was added to 2 µL of 0.1% poly-L-lysine in 10 mM Tris, pH 8.3. The sample was allowed to interact for 1 h and then 1 µL was added to ROX-labeled DNA/formamide. The chemical structure of poly-L-lysine is shown above the electropherogram.
Figure 9-23. Electropherograms obtained on an ABI 310. 1 µL of 0.05 mM CoCl\textsubscript{2} in 10 mM Tris, pH 8.3 was added to 11.5 µL of ROX-labeled DNA/formamide and 0.1 µL of 0.05 mM TOTO-3 dye (diluted in dH\textsubscript{2}O) was added to 24 µL of ROX-labeled DNA/formamide. The samples were allowed to interact for 1 h and then they were measured. The chemical structure of TOTO-3 is shown in the TOTO-3-ROX-labeled DNA run.
intercalating dyes that caused negative CE effects, TOTO-1 and TOTO-3 have very similar structures. They consist of symmetric dimers of cyanine dyes with four positive charges while TOPRO-3 is a monomeric dye with two positive charges. It was found that the concentration of this monomeric dye had to be approximately three orders of magnitude greater than the concentration of either of the dimeric dyes to yield similar peak broadening. The large difference in concentrations needed to cause negative effects for the dimeric vs. monomeric dyes is likely the result of the improved binding affinities of the dimeric dyes.\textsuperscript{154}

\subsection*{9.2.3.9. Conclusions}

Overall, these studies reveal the detrimental role that certain transition metal cations can have on DNA typing. These cations may be present in locations where environmental or forensic samples are collected. Zinc, cobalt, and nickel exist in the air, soil, and water, coming from both natural and industrial sources and can also be found at elevated levels in certain biological fluids.\textsuperscript{155,156} It was found that these cations cause broadened, low intensity peaks with mobilities shifted from their normal B-DNA counterparts. The resulting samples fail normal quality control procedures, producing poor quality electropherograms and inadequate calibrations. However, simple solutions to reverse these effects by utilizing either a chelating agent or pH adjustment of the sample, were demonstrated. It is interesting to note that in slab-gel DNA sequencing applications, samples were typically reconstituted in a formamide-EDTA mixture after an ethanol precipitation.\textsuperscript{157,158} But as capillary array electrophoresis became the preferred
technology, EDTA was eliminated from the formamide loading solution to increase the electrokinetic injection efficiency.\textsuperscript{158}

The extent of these poor results was very much dependent on the DNA sample. For example, the concentration of cations necessary to cause similar broadening effects in different DNA samples varied, most likely due to a combination of factors including the DNA fragment size and sequence and the concentration of DNA in the solution exposed to the metal ions. Furthermore, the length of time that the sample remained in formamide before CE analysis was also a very important factor in the degree of peak broadening and shifted mobility.

The broad allele and ladder peaks present in the CE runs of M-DNA samples indicate that these affected fragments have either a range of properties (molecular mass, charge, and/or structure), the fragments have aggregated, or an injection problem is occurring. However, if the broadness was the result of an injection problem stemming from the higher salt concentration, one would expect the same result for samples exposed to the metal ions at pH 7. Additionally, if clumping of the fragments was occurring, the alleles would migrate at a much lower mobility. Therefore, the existence of higher mobility peaks indicates that these CE results are due to a structural change in the DNA upon metal binding and not just a consequence of aggregation or the altered charge and mass of the molecule. The ssDNA-metal complexes must exist in a more condensed form than normal.

Of the other materials added to the DNA samples, only the polycationic species, spermine and poly-L-lysine, and the intercalating dyes caused mobility shifts or loss of
resolution in the resulting separations. The loss of peak resolution and altered migration rate for the dye-bound DNA samples were in agreement with previous work on dye intercalated double stranded DNA by Kim and Morris\textsuperscript{159} and Zhu and colleagues.\textsuperscript{160} These researchers demonstrated that the electrophoresis results were due to an inhomogeneous number of dye molecules distributed through each DNA fragment, leading to multiple DNA/dye conformations and DNA/dye mass-to-charge ratios, each with a slightly different persistence length and mobility.

\textbf{9.2.4. Atomic Force Microscopy Results}

To gain additional information about the structure of M-DNA, including its diameter and aggregative behavior, AFM was utilized. Since the sample is adsorbed onto the mica, the conformation or structure of the DNA is inevitably modified by the transition from a three dimensional structure in solution to a two dimensional form on a mica surface. However, comparisons of the two dimensional forms of B-DNA and M-DNA can at least offer some indication of the structural differences that also exist in solution.

In agreement with the CE results, AFM studies demonstrated that a significant structural difference exists between M-DNA and normal DNA samples without metal ions bound to the nucleobases. Furthermore, these M-DNA samples often formed extensive self-assembled patterns on mica. Figure 9-24 depicts representative images of B-DNA while Figures 9-25 to 9-33 depict various M-DNA patterns on mica. These interesting structures were not observed in B-DNA samples or DNA samples that were
Figure 9-24. AFM images of B-form λ-DNA. A representative set of images on different mica substrates and at different DNA concentrations.
exposed to the same divalent cations but in buffer solutions with a lower pH, 7, (where
the cations can only interact with the phosphate backbone, not the nucleobases). In
addition, these patterns were never detected when blank samples (divalent cations in pH
8.3 buffer without λ-DNA) were added to mica surfaces.

As shown, B-DNA adsorbed on mica typically displays a randomly coiled and
tangled structure or when the concentration of the molecules is large enough, a regular,
mesh-like DNA network. On the other hand, the DNA samples exposed to Zn, Co, and
Ni in a buffer at pH 8.3, often formed “neuron-like” patterns as shown in Figure 9-25.
Specifically, these samples displayed bundles or thick clumps with emerging branched
ropes. The number of DNA molecules contained in these ropes ranged from a few tens
up to thousands (as estimated from the topographic profile).

Besides the neuron-like patterns observed with all of the transition metals, the
other self-assembled forms appeared to be dependent on the cation identity. For
example, a knot and rope network as shown in Figure 9-26 was only found for Ni-λ-DNA
The knots were linked through bundled and individual DNA fibers with some knots
having up to six fibers radiating outwards. On some areas of the mica, the DNA bundles
in these networks were also found to be very linear (upper images in Figure 9-26). This
result has to be due to the metal ions intercalated into the DNA because no alignment or
stretching procedures were utilized and these areas were observed in the center of the
mica disc. The absence of a receding droplet edge at this location excludes alignment by
the slight drying of the droplet within the 10 min it was exposed to the mica substrate.
Figure 9-25. (a) and (b) AFM images of Ni-λ DNA on a mica substrate. (c) AFM image of Co-λ DNA on a mica substrate.
Figure 9-26. AFM images of Ni-λ DNA on a mica substrate. Arrow points to a knot from which 5 DNA fibers radiate and one fiber passes through.
Other interesting patterns observed on mica for Ni-λ-DNA are depicted in Figure 9-27. As these images show, some of the M-DNA existed as very linear strands and rod-like bundles. The rods were not oriented in any particular direction and therefore, these structures cannot be attributed to alignment and stretching of the DNA on the mica surface. Frequently these rods were also observed to assemble into hexagonal patterns. Some of these patterns are marked with arrows on the images shown.

The most elegant Ni-λ-DNA assemblies, “snowflake-like” patterns were only observed twice out of approximately fifty Ni-λ-DNA applications to various mica substrates, with 10-20 AFM scans of different areas on each of these substrates. Representative images of these patterns are shown in Figure 9-28. The size of these fractal structures varied widely as did the number of arms on each. It is interesting to note that other researchers detected very similar fractal DNA structures after their samples had been UV-irradiated or denatured.161,162 Chandra et al. found that under irradiation, calf thymus DNA degraded into fragments of different sizes and these fragments aggregated into fractal-like structures upon drying on a glass surface.161 Yan and Iwasaki also demonstrated that denatured plasmid DNA aggregated together to form fractal structures on mica, following the diffusion limited aggregation (DLA) model.162 In the DLA model it is assumed that particles are added one at a time to a growing aggregate of identical particles.163 In this model, particles that originate at some distance from the seed of the aggregation perform a random walk to encounter the aggregate and subsequently attach to the position where they first contact the cluster. The cluster’s
Figure 9-27. (a), (b), (c), and (d) AFM images of Ni-\(\lambda\) DNA on a mica substrate. Arrows point to hexagonal patterns within the images. (d) A closer scan of the hexagonal structure in (b).
Figure 9-28. AFM images of Ni-λ-DNA snowflake patterns formed on mica substrates.
fractal structure arises because the faster growing tips of the aggregate shield the other regions from incoming particles. Figure 9-29 displays a variation of this snowflake pattern that was observed on another Ni-λ-DNA sample on mica. These snowflake structures were more globular and generally had less branching than those depicted in Figure 9-28. Furthermore, they appeared to be forming within or on top of much larger patterns. Figure 9-30 shows additional representative images of these larger networks at varying magnifications. These patterns show a much different symmetry than the snowflakes, which possess branches radiating from the center in all directions.

Finally, “fern-like” fractal aggregates were also observed. These patterns consisted of self-similar branching structures that ranged in size from a few to hundreds of micrometers and were only found when λ-DNA samples were metallated with Co²⁺ (Figure 9-31). It was noted that often these fern-like structures remained undeveloped, with only a few branches. While snowflake and fern-like fractal patterns were observed for Ni-λ-DNA and Co-λ-DNA, respectively, fractal patterns were never found on mica substrates with Zn-λ-DNA samples.

These AFM results for Ni-λ-DNA, Co-λ-DNA, and Zn-λ-DNA were in contradiction with observations on M-DNA made by Moreno-Herrero et al.¹⁶¹ These researchers found no major difference between the Ni²⁺ and Co²⁺ M-DNA that they examined, nor did they observe any fractal patterns. It is likely that the difference in results is due to the lower quantity of divalent cations that their DNA samples were exposed to. For example, while they exposed a concentration of ~15 nM pUC18 DNA (2,686 bp) to 1 mM Co²⁺, a concentration of ~120 pM λ-DNA (48,502 bp) was exposed
Figure 9-29. AFM images of Ni-λ-DNA globular snowflake structures on top of much larger patterns, formed on mica substrates.
**Figure 9-30.** AFM images of Ni-λ-DNA large, network-like patterns formed on mica substrates.
Figure 9-31. AFM images of Co-λ-DNA fern-like patterns formed on mica substrates.
to 3 mM Co$^{2+}$. Therefore, while Moreno-Herrero et al. had less than a 10-fold excess of divalent cations to DNA binding sites (phosphate groups and base pairs), a 150-fold excess was present in the $\lambda$-DNA experiments. Using the fluorescence assay, it was established that this ratio of divalent cations to DNA base pairs was necessary for maximum reduction in TOTO-1 fluorescence intensity, i.e. maximum metal intercalation in the $\lambda$-DNA strands.

In addition to these unusual patterns, it was determined that the M-DNA samples often had strand heights that were much greater than the B-DNA samples. This result is in agreement with previous work$^{48,164}$ and is indicative of the individual DNA strands existing in a condensed state or the presence of multiple DNA strands forming a bundle. A representative topographic measurement of B-DNA height is shown in Figure 9-32 and topographic measurements of various M-DNA forms are given in Figures 9-33 through 9-36. These heights were found to range from 1-2 nm for B-DNA and from 8-50 nm for M-DNA. As mentioned previously, the diameter of B-DNA is expected to be 2 nm, but because of the adsorption of the molecule to the surface, the typical heights measured are less than 2 nm. However, the widths measured for B-DNA strands are much larger (approximately 30 nm) due to exaggeration caused by the size of the AFM tip radius.

Unfortunately, gaining control over the formed pattern proved difficult to achieve. It was found that even when the same DNA sample was placed on two pieces of mica from the same lot, under what seemed to be identical conditions (humidity, temperature, length of time on the surface, etc.) the structures formed would often be different. After spending some time trying to control parameters that might be affecting the DNA
**Figure 9-32.** Topographic height profile of B-form λ-DNA taken along the line on the AFM image. As shown, a typical height measured was 1.5 nm.
Figure 9-33. Topographic height profile of rod-like Ni-λ-DNA taken along the line marked on the AFM image. As shown, a typical height measured was ~28 nm.
Figure 9-34. Topographic height profile of snowflake-like Ni-λ-DNA taken along the line marked on the AFM image. As shown, a typical height measured was 49 nm.
Figure 9-35. Topographic height profile of fractal Ni-λ-DNA pattern taken along the line marked on the AFM image. As shown, a typical height measured was 25 nm.
Figure 9-36. Topographic height profile of fern-like Co-λ-DNA structures taken along the line marked on the AFM image. As shown, a typical height measured was 12 nm.
formation, it was concluded that the varying results were likely due to the way the droplet spread on the mica surface. When a droplet of DNA is added to a freshly cleaved mica surface, the solution spreads rapidly across the substrate in a thin layer because of the high hydrophilicity of the mica surface. However, it was found that slight imperfections in the mica smoothness typically led to some inhomogeneities in the droplet spreading pattern. It was near these imperfections that the more rare structures, including the fern-like Co-λ-DNA, would often form. For example, Figure 9-37 shows an AFM image of a linear edge on the mica surface where the Co-λ-DNA structures have abruptly ended. Beyond this edge, Co-λ-DNA fern-like patterns were not observed.

9.2.5. Conclusions

Overall, AFM together with CE and fluorimetry studies revealed that the M-DNA samples examined were condensed but not aggregated unless placed on a surface. Interestingly, when the samples came into contact with mica substrates, intricate patterns were formed. These self-assembled structures might be applicable in the fabrication of future nanoelectronic devices. In solution, the condensed M-DNA molecules are likely less flexible and therefore, not as easily stretched as B-DNA. For this reason, larger ac fields or shorter device gap lengths will need to be utilized for maximum attachment across the electrodes. More importantly, these structural studies have revealed information that is relevant to the use of M-DNA as a molecular wire and/or as a building material in any future nanoscale application.
Figure 9-37. AFM images of Co-λ-DNA on a mica substrate. The image on the left shows evenly distributed fractal patterns while the image on the right shows the edge of this fractal pattern, beyond which structurally similar designs were not observed.
Chapter 10. Repetitive DNA Synthesis and Characterization

Previous theoretical and experimental research has shown that highly repetitive DNA may provide for better electrical transport than a random sequence like λ-DNA. Therefore, a method for the synthesis of repetitive DNA fragments with lengths sufficient to span fabricated electrode gaps has been developed. Short repetitive oligonucleotides (up to 20 base long poly G and 40 base long poly A, poly T, and poly C) are commercially available from companies performing DNA synthesis using the typical phosphoramidite chemistry. However, the lengths of these fragments are only between 7 and 14 nm. Utilizing the method described in Section 2.17.1, these short DNA fragments could be enzymatically ligated to lengths >1000 bp or ~340 nm.

The ligation procedure was adapted from Sasagawa et al. These researchers synthesized (CTG)$_7$ and (CAG)$_7$ oligonucleotides and obtained (CTG/GAC)$_{46}$ repeat DNA by annealing and artificial ligation with T4 DNA kinase and T4 DNA ligase. However, a slightly different procedure had to be utilized to ligate perfectly repetitive sequences. To accomplish this, T4 DNA polymerase was introduced after initial hybridization of the complementary strands. These complementary strands do not all anneal end to end because of the nature of the sequence. By using T4 DNA polymerase, the duplex DNA starting material was made blunt-ended and therefore, upon ligation, repetitive DNA sequences were created without gaps or overhanging ends. To the author’s knowledge, this is the first enzymatic method developed to synthesize long poly(A/T), poly(G/C), and poly(AT/TA) without any defects or gaps present in the duplex.
10.1. Capillary Electrophoresis Characterization of Ligation Products

The resulting product of each step of the ligation procedure was characterized using native capillary electrophoresis with PVP as the sieving matrix. The CE separation of a pBR 322/Hae III digest of known fragment lengths was utilized for sizing. Figure 10-1 displays the electropherogram of the digest with the corresponding fragment sizes listed above the peaks. Figure 10-2 (a) displays the electropherogram obtained after hybridization of the starting material, 40 base single stranded poly(A) annealed to 40 base single stranded poly(T). The presence of multiple peaks is due to the imperfect nature of hybridization for repetitive sequences. If the DNA is run under denaturing conditions, a single peak is present. Figure 10-2 (b) shows the electropherogram of the blunt-ended, double stranded product that results from treating the hybridized material shown in Fig. 10-2 (a) with T4 DNA polymerase. This enzyme catalyzes the 5’ to 3’ synthesis of DNA and also possesses 3’ to 5’ exonuclease activity. Therefore, after treatment with the polymerase, strands that were both shorter and longer than the starting hybridized material were formed. Figure 10-2 (c) depicts the electropherogram of a typical product yielded from the addition of T4 DNA ligase to the blunt-ended material shown in Figure 10-2 (b). The inset in this figure shows an enlarged region of the electropherogram for a clearer view of the multiple fragment lengths present. This particular ligation yielded a mixture of products up to approximately 745 bp in length.

10.2. Effect of Polymer on Ligated Length

It was found that the addition of PEG 4000 to the ligation reaction mixture increased not only the ligase activity and therefore, the length of the ligated product but
Figure 10-1. Electropherogram of pBR 322/Hae III digest, separated using 5.5% PVP in TBE buffer and YO-PRO-1 dye mixed with the DNA sample. Fragment size (in bp) shown above each peak.
Figure 10-2. (a) Electropherogram of hybridized starting material (40 base single stranded poly(A) annealed to 40 base single stranded poly(T)). (b) Electropherogram of the blunt-ended hybridized material. (c) Electropherogram of the product yielded after the addition of T4 DNA ligase to material shown in (b). Inset depicts expanded view of electropherogram which more clearly illustrates the multiple peaks present. All separations were completed using 5.5% PVP in TBE buffer with YO-PRO-1 in the run buffers and sieving matrix.
also the ligation efficiency of certain fragment lengths. For example, using this polymer large fractions of the poly(AT/TA) product ligated to ~1500 bp could be obtained while the other ligation lengths in the mixture were present in much smaller amounts. Figure 10-3 displays the electropherogram obtained after this ligation. Using PEG 4000, large fractions of ~600 bp poly(G/C) and ~560 bp poly(A/T) were also obtained. In addition, other polymers like PEG 6000, polyacrylamide, and PVP had a similar effect on the ligation efficiency.

As for the stimulation of ligation by introducing macromolecules like PEG, previous research has demonstrated that this is due to the polymers causing crowding in the solution.\textsuperscript{101,102} By excluding volume and therefore, bringing more DNA termini in contact with one another, polymers have been shown to improve the activity of T4 DNA ligase by ~1000-fold.\textsuperscript{102} It is unclear why certain product lengths appear to be favored. If this is an effect of the polymer serving as a template for ligation, one would expect roughly the same length to be preferred, irrespective of the DNA sequence. However, as discussed earlier, this is not the case.

10.3. Confirmation of Thiol Labeling

Ligated repetitive DNA fragments could be thiol-labeled for attachment to gold electrodes by utilizing T4 polynucleotide kinase to transfer a thiophosphate from ATP\textsubscript{γ}S to the 5′-OH group as described in Section 2.17.2. Figure 10-4 displays the results from the fluorimetry assay used to confirm successful labeling. It was found that these thiol-labeled samples typically adsorbed to the Au-coated strip with ~80% efficiency.
Figure 10-3. (a) Electropherogram of the blunt-ended hybridized poly (AT/TA). (b) Electropherogram of the product yielded after the addition of T4 DNA ligase and 50% (w/v) PEG 4000 to material shown in (a). Note the time scale differences between the two electropherograms. The arrow points to the remaining blunt-ended material that was not ligated. All separations were completed using 3% PVP in TBE buffer with YO-PRO-1 in the run buffers and sieving matrix.
Figure 10-4. Representative fluorimetry results using Hoechst 33258 dye for characterization of thiol-labeling efficiency. The plot shows fluorescence spectra collected before and after the addition of a Au-coated strip to thiol-labeled poly(A/T) DNA.
10.4. High Performance Liquid Chromatography Separation and Collection

A HPLC and fraction collection method using an Eclipse DNA column was developed to separate and obtain the desired repetitive DNA fragment from the ligation reaction mixture. In this way, the current-voltage characteristics of only one length of DNA can be measured. Figure 10-5 displays a chromatogram obtained for an injection of the pBR 322/Hae III restriction fragment mixture. This standard sample was used prior to injecting and collecting ligated mixtures to assure the quality and reproducibility of the HPLC separation. Figure 10-6 (a) shows the resulting chromatogram for the injection of a thiol-labeled poly(G/C) mixture. During this run a Gilson Fraction Collector was set to collect in time mode and as displayed in the chromatogram of Figure 10-6 (b), the desired fragment length (~600 bp) was successfully obtained.

In summary, an enzymatic method for the synthesis of perfectly repetitive duplex DNA sequences without defects was developed. A CE method was used for the characterization of the strand lengths in the resulting ligation mixtures. Furthermore, an HPLC method for the separation of double stranded DNA and the collection of the desired fragment was established. The ligated lengths of these collected fragments are compatible with the electrode gap sizes that can be fabricated.
Figure 10-5. Separation of pBR 322/Hae II digest (0.37 µg) using an Eclipse column (4.6 x 75 mm). To separate the DNA, two eluents were used, eluent A: 0.1 mM TEAA/0.1 mM EDTA in water and eluent B: 0.1 mM TEAA/0.1 mM EDTA in 25% acetonitrile, pH 7. The HPLC separation method utilized a gradient of 40-80% eluent B in 30 min at a flow rate of 1.0 ml/min and a temperature of 45 °C. Fragments were detected by their UV absorbance at 260 nm. The sizes of the DNA fragments are given in base pairs.
Figure 10-6. Separations completed using an Eclipse column with a gradient of 40-80% eluent B in 30 min at a flow rate of 1.0 ml/min and a temperature of 45 °C. Fragments were detected by their UV absorbance at 260 nm. (a) 2 µL injection of poly(G/C) ligation mixture. (b) 8 µL injection of the collected fragment from (a) after concentrating the sample using a Microcon filter. Fraction collection performed in time mode.
Chapter 11. Conclusions and Future Research

11.1. Conclusions

A reproducible method for the alignment and attachment of disulfide-labeled λ-DNA molecules to Au electrodes was developed. \( I-V \) measurements of λ-DNA molecules and macroscopic DNA fibers demonstrated that the double helix with its corresponding base pair stack is necessary for sizable electrical conduction and that conduction due to other pathways is minimal. The room temperature resistance estimated for λ-DNA in its native, undamaged state is 10 TΩ. While this value is well above the resistance of other possible interconnect candidates in nanoelectronics like carbon nanotubes (~100 kΩ), DNA should still be considered an important tool in this field because of the inherent advantages that it possesses. Its unique self-assembly capabilities resulting from the highly specific hybridization of complementary DNA strands has clearly been demonstrated by Seeman and colleagues.\(^{167-169}\) In addition, the ability to manipulate the \( I-V \) characteristics of λ-DNA through the alteration of its structure, points to its promise to be electronically functionalized.\(^{71,72}\) Specifically, nicks can be introduced into the phosphate backbones of the DNA duplex to yield an additional low-field resistance or a high-field rectification effect. Furthermore, by increasing the quantity of these nicks or by single stranding the molecule, its conductivity can be lowered by two to three orders of magnitude. Conversely, DNA conductivity can be improved by repairing damage with T4 DNA ligase. These results indicate that DNA’s well-studied enzymatic chemistry can be utilized to selectively manipulate the molecule’s
structure either prior to or after integration into a circuit to yield the desired electronic behavior.

Preliminary results in this dissertation also demonstrate that the conductivity of this molecule can be improved. Insertion of nickel ions into the base pairs of λ-DNA with two nicks caused the conductivity gap in the $I-V$ curve to disappear or at least to decrease to a length that could not be observed. However, the parameters necessary for maximum M-DNA strand alignment across the electrode gaps still need to be optimized. This is likely due to structural changes (observed by CE and AFM) that occur upon DNA metallation.74

Theoretical work indicates that DNA sequences where all base pairs are identical should yield an ideal periodic potential for charge transport.61 To experimentally examine the effect of base content, a multistep enzymatic procedure for the synthesis of long (>600 bp) sequences of poly(AT/TA), poly(G/C), and poly(A/T) has been developed. This method yields blunt-ended, thiol-labeled repetitive duplexes without any defects. With further study, it should be possible to use electronically functionalized M-DNA with perfectly repetitive sequences, to produce structures with improved charge transport properties.

11.2. Future Research

Additional $I-V$ measurements of metallated λ-DNA (Ni-λ, Zn-λ, and Co-λ) and λ-DNA with various counterions (exchanged using the optimized dialysis method) are required to more thoroughly explore the effect of these cations. $I-V$ characteristics of the λ-DNA sample synthesized with a single base pair mutation (Section 2.2.2), should also
be examined to determine the influence (if any) of such defects. Furthermore, once devices with electrode gaps of ~100-200 nm are fabricated, repetitive, thiol-labeled sequences can be measured.

It would also be interesting to explore the effect of the DNA molecule’s conformation on the resulting I-V collected. At least six different DNA isomers (designated A, B, C, D, E, and Z) have been identified. However, only A, B, and Z-DNA conformations have been found in nature. Direct electrical conductivity measurements of DNA thus far have focused on the B or A-form. A conversion between these two conformations can be induced by either adjusting the ionic strength of the solution or by dehydrating the DNA. It would be worthwhile to explore the electronic properties of other conformations of disulfide-labeled λ-DNA. For example, the C-motif which possesses ~9 bp per turn and a 19 Å diameter can be induced by adding the cationic lipid, dimethyldioctadecylammonium bromide to B-DNA. One would expect that this conformational adjustment would induce a change in the gap between the HOMO and LUMO orbitals and therefore, cause a change in the molecule’s transport characteristics. Other possibilities for inducing conformational changes include the exposure of B-DNA to [Co(NH$_3$)$_6$]$_3^+$ which causes a B→Z transition. Z-DNA is a left-handed helix with 12 bp per turn and a diameter of 18 Å. Furthermore, in addition to chemicals, certain proteins can induce conformational transitions. For example, RecA has been shown to cause a structural change in the B-DNA helix from its normal 10 bp to ~18 bp per turn. By adding these compounds, in their necessary buffer solutions, the conformation of λ-DNA molecules could be adjusted after alignment on the device.
Another possible area to investigate is the influence of various intercalating dyes on the DNA molecule’s electronic properties. Dyes like TOTO-1, have either one or two fluorophores which can intercalate between the base pairs and therefore, these compounds could change the molecule’s natural electrical conductivity. TOTO-1 binds without base specificity but by comparing intercalating dyes that do possess different sequence binding affinity one could gain additional information about the mechanism of hole or electron transport. For example, the dye, ethidium homodimer II intercalates with an A/T bp preference while 7-aminoactinomycin D selectively binds to G/C bp.\textsuperscript{175}

In closing, despite all of the recent experimental studies on DNA conductivity there are still many relatively unexplored areas. Of particular interest is the development of methods to engineer both levels and types of DNA conductivity. Clearly, if this can be accomplished, DNA will prove to be an extremely useful material for nanoelectronics.
References


Appendices

A. Poly(dimethylacrylamide) Synthesis


1. Distill $N,N$-dimethylacrylamide (DMA) under reduced pressure to remove the stabilizer. DMA should distill at $\sim 30 \, ^{\circ}\text{C}$ under a vacuum of 0.1 mm Hg.

2. Mix 65 mL of methanol with 185 mL of deionized water and 25 g of distilled DMA.

3. Bubble nitrogen through the solution for 2 h.

4. Add 1.25 mL of ammonium persulfate (APS) stock solution (made by dissolving 0.2 g of APS in 1.8 mL of deionized water) to the mixture.

5. Add 1.25 mL of $N,N,N,N$-tetramethylethylenediamine (TEMED) stock solution (made by dissolving 260 $\mu$L of TEMED in 1.8 mL of deionized water) to the mixture.

6. Stir mixture with magnetic stir bar for 36 h under ambient conditions.

7. Precipitate poly(dimethylacrylamide) (PDMA) by adding 600 mL of cold acetone (5-10 $^{\circ}\text{C}$) to the solution. Only the high molecular weight ($\sim$1 M) fraction of polymer precipitates readily from cold acetone.

8. Decant aqueous layer and use a rotary evaporator to pull off remaining liquid.

9. Make a 7.3% PDMA (w/v) solution in 100 mM TAPS, pH 8 with 8 M urea, and 5% 2-pyrrolidinone.
B. D1S80 Synthesis and Characterization

B.1. DNA Extraction from FTA® Paper


1. Remove a 1 mm disc from a bloodstain sample on FTA® paper using a small puncher.

2. Place the disc in a PCR amplification tube.

3. Add 200 µL of FTA purification reagent (Whatman Bioscience Inc.) to the tube.

4. Cap tube and vortex for 3 s at low speed.

5. Incubate sample for 5 min at ambient conditions with a second vortex halfway through the incubation.

6. After 5 min incubation, vortex a third time and then remove all FTA purification reagent.

7. Repeat steps 3-6 an additional two times for a total of three washes with the reagent.

8. Add 200 µL of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0).

9. Cap tube and vortex for 3 s at low speed.

10. Incubate sample for 5 min at ambient conditions with a second vortex halfway through the incubation.

11. After 5 min incubation, vortex a third time and then remove all TE buffer.

12. Repeat steps 8-11 one additional time.

13. Allow punch to dry at ambient conditions for about 1 h before performing PCR.

14. Add PCR amplification mix directly to the punch containing the purified immobilized DNA.
B.2. PCR Amplification

Note: Promega PCR Master Mix (#M7502) which contains Taq DNA polymerase, dATP, dGTP, dCTP, dTTP, and MgCl₂ in a proprietary reaction buffer was used for amplification. Procedure from Promega Corporation. PCR Master Mix Protocol. Madison, WI, 2001. Primers were ordered from IDT without modifications or with either a 5’ C6 S-S or a 5’ C6 NH₂ modifier. Disulfide-labeled primers were dissolved in a solution of 0.05 M DTT to reduce the functional groups to thiols. Amplification was performed using a GeneAmp® PCR System 9700 thermal cycler.

Forward primer: 5’ GAAACTGGCCTCCAAACACTGCCCGCCG 3’
Reverse primer: 5’ GTCTTTGTTGGAGATGCACGTGCCCCTTGC 3’

1. Thaw PCR Master Mix at room temperature.
2. Vortex the Master Mix and then spin briefly in centrifuge.
3. Prepare the following reaction mix on ice: 12.5 µL of 2× PCR Master Mix, 1 µL of 10 µM forward primer, 1 µL of 10 µM reverse primer, DNA template (FTA disc), and 10 µL of nuclease-free water.
4. Perform PCR using a thermal cycler set with the following conditions: a) 2 min denaturation at 95 °C, b) 1 min annealing step at 65 °C, c) 1 min extension step at 72 °C (with a final extension of 5 min at 72 °C), and d) a final hold at 4 °C. 28 cycles resulted in optimal amplification of desired product.

B.3. Capillary Electrophoresis on Beckman MDQ Using 7.3% PDMA

Note: The sieving matrix consisted of 7.3% PDMA (as described in Appendix A) and with this polymer solution, 100 mM TAPS, pH 8.0 was used as the run buffer. Detection was accomplished using the intercalating dye, YO-PRO-1 iodide and a 488 nm Ar-ion laser.

1. Add 1 µL of 1 mM YO-PRO-1 per 5 mL of run buffer and sieving matrix solution. Vortex solutions to mix evenly and sonicate for 1 min to remove any bubbles that have formed.
2. Dilute DNA sample in formamide and maintain the capillary cartridge temperature at 55 °C to separate DNA under denaturing conditions.
3. Rinse 41 cm long capillary (50 µm internal diameter) with run buffer for 2 min at 75 psi.
4. Fill capillary with sieving matrix by rinsing for 5 min at 75 psi.
5. Inject sample electrokinetically by applying 10 kV for 5 s.
6. Separate sample using 10 kV for 30 min with reverse polarity.

B.4. Capillary Electrophoresis on ABI 310 Using an Intercalating Dye

1. Add 0.2 µL of 1 mM TOTO-1-iodide to 50 µL of deionized water and vortex to mix evenly.

2. Add 1 µL of amplified sample to this solution and vortex to mix evenly.

3. Let sample sit at ambient conditions for 10 min.

4. Inject, separate, and detect sample on an ABI 310 using standard run conditions.
C. Microcon Filtration

Note: Microcon YM-100 filters (#42412) were obtained from Millipore. The following procedure is adapted from: Millipore Corporation. Microcon Centrifugal Filter Devices User Guide. Bedford, MA, 2000.

1. Insert the blue Microcon sample reservoir into vial.

2. Pipette sample solution into sample reservoir (500 µL maximum) without touching the membrane.

3. Seal with attached cap and align the cap strap toward the centrifuge rotor.

4. Centrifuge cartridge for 14 min at 500 × g (~2700 RPM), while using a compatible counterbalance. The centrifugal force can be calculated using this formula: \( RCF = 1.118 \times 10^{-6} \times \text{radius} \times (\text{RPM})^2 \) where the radius is the distance in millimeters from the center of rotation to the base of the filtrate vial.

5. Remove assembly from centrifuge and place sample reservoir upside down in a new vial.

6. Spin for 4 min at 1000 × g (~3900 RPM) to transfer retentate to vial.
D. Synthesis of Repaired and Nicked λ-DNA with 3’ Disulfide Labels on Opposite Strands

Note: λ-phage DNA from Sigma (#D9768) was diluted to 10 nM with deionized water. T4 polynucleotide kinase and its 10× reaction buffer (#EK0031), T4 DNA ligase and its 10× reaction buffer (#EL0334), and 100 mM ATP (#R0441) were obtained from Fermentas. The ATP solution was diluted to 0.1 mM with deionized water. Shrimp alkaline phosphatase (SAP) with its 10× reaction buffer was purchased from Promega (#M820A). Microcon YM-100 filters were obtained from Millipore (#42413). Labeled oligonucleotides were ordered from IDT with 3’ C3 S-S modifiers and were diluted to a concentration of 1.4×10⁻⁴ M.

λ1: 5’ AGGTCGCCGCCC 3’
λ2: 5’ GGGCGGCGACCT 3’

1. Phosphorylate λ1 at its 5’ end using T4 polynucleotide kinase: Take 3 µL of λ1 (1.4×10⁻³ M) and add 20 µL of 10× reaction buffer A, 2 µL ATP (0.1 mM), and 8 µL T4 polynucleotide kinase (10 U/µL). Dilute to a final volume of 200 µL with deionized water and incubate at 37 °C for 1 h.

2. Repeat step 1 with 3 µL of λ2 (1.4×10⁻⁴ M).

3. Remove 5’ phosphates from λ-DNA using SAP: Take 40 µL of λ-DNA (10 nM) and add 5 µL of 10× SAP reaction buffer and 1.5 µL SAP enzyme (1 U/µL). Dilute to a final volume of 50 µL with deionized water and incubate at 37 °C for 15 min. Inactivate SAP by heating at 65 °C for 15 min.

4. Hybridize phosphorylated λ1 to dephosphorylated λ-DNA by mixing the two solutions (there is a 10³-fold excess of λ1) and heat to 75 °C and then cool slowly to room temperature by turning off the water bath or thermal cycler.

5. Ligate λ1 to λ-DNA by adding 40 µL of 10× ligation buffer and 4 µL of T4 DNA ligase (5 U/µL) to the solution from step 4. Vortex and spin down and then incubate at 22 °C for 12 h.

6. Pass solution from step 5 through a YM-100 filter to remove excess λ1.

7. Dilute λ-DNA/λ1 retentate from step 6 to 50 µL with deionized water and hybridize and ligate phosphorylated λ2 to this molecule, following steps 4 and 5.

8. Pass solution from step 7 through a YM-100 filter to remove excess λ2.
9. Dilute retentate according to quantitation assay to give a final concentration of 5 nM disulfide-labeled λ-DNA.

10. To prepare λ-DNA with two nicks in its phosphate backbone, the above procedure is followed. However, after the hybridization of each of the complementary oligonucleotides (λ1 and λ2) to the λ-DNA template, the phosphate backbones are left incomplete, i.e. ligation by T4 DNA ligase is not performed. The hybridization starting temperature is also lowered to 65 °C when annealing λ2 to the λ-DNA template to prevent the loss of λ1 from the template since it is not ligated into place.
E. Synthesis of Repaired and Nicked λ-DNA with Disulfide Labels on the 5' and 3' Ends of the Same Strand

Note: λ-phage DNA from Sigma (#D9768) was diluted to 10 nM with deionized water. T4 polynucleotide kinase and its 10× reaction buffer (#EK0031), T4 DNA ligase and its 10× reaction buffer (#EL0334), and 100 mM ATP (#R0441) were obtained from Fermentas. The ATP solution was diluted to 0.1 mM with deionized water. Microcon YM-100 filters were obtained from Millipore (#42413). Oligonucleotides were ordered from IDT. λ2 and λ3 were labeled with a 3' C3 S-S and a 5' C6 S-S modifier, respectively. λ extension was left unlabeled. Each of these oligonucleotides was diluted to a concentration of 1.4×10^{-4} M.

λ2: 5' GGGCGGCGACCT 3'
λ extension: 5' AGGTCGCCGCCCATGGCATGGACGC 3'
λ3: 5' GCGTCCATGCCAT 3'

1. Phosphorylate λ2 at its 5' end using T4 polynucleotide kinase: Take 3 µL of λ2 (1.4×10^{-4} M) and add 20 µL of 10× reaction buffer A, 2 µL ATP (0.1 mM), and 8 µL T4 polynucleotide kinase (10 U/µL). Dilute to a final volume of 200 µL with deionized water and incubate at 37 °C for 1 h.

2. Repeat step 1 with 3 µL of λ extension (1.4×10^{-4} M).

3. Hybridize phosphorylated λ2 to λ-DNA by mixing the solution from step 1 with 40 µL of λ-DNA (10 nM) (there is a 10^3-fold excess of λ2). Heat solution to 75 °C and then cool slowly to room temperature by turning off the water bath or thermal cycler.

4. Ligate λ2 to λ-DNA by adding 40 µL of 10× ligation buffer and 4 µL of T4 DNA ligase (5 U/µL) to the solution from step 3. Vortex and spin down and then incubate at 22 °C for 12 h.

5. Pass solution from step 4 through a YM-100 filter to remove excess λ2.

6. Dilute λ-DNA/λ2 retentate from step 5 to 50 µL with deionized water and hybridize and ligate phosphorylated λ extension to this molecule, following steps 3 and 4.

7. Pass solution from step 4 through a YM-100 filter to remove excess λ extension.

8. To λ-DNA/λ2/λ extension retentate from step 7 add 3 µL of λ3 (1.4×10^{-4} M). Dilute to a final volume of 200 µL with deionized water. Hybridize and ligate λ3 to the template by following steps 3 and 4.
9. Pass solution from step 8 through a YM-100 filter to remove excess \( \lambda_3 \).

10. Dilute retentate according to quantitation assay to give a final concentration of 5 nM disulfide-labeled \( \lambda \)-DNA.

11. To prepare \( \lambda \)-DNA with three nicks in its phosphate backbone, the above procedure is followed. However, after the hybridization of each of the complementary oligonucleotides (\( \lambda_2 \), \( \lambda \) extension, and \( \lambda_3 \)) to the \( \lambda \)-DNA template, the phosphate backbones are left incomplete, i.e. ligation by T4 DNA ligase is not performed. The hybridization starting temperature is also lowered to 65 °C when annealing \( \lambda \) extension and \( \lambda_3 \) to the \( \lambda \)-DNA template. This is used as a precaution to avoid the de-hybridization of the previously annealed oligonucleotides (since they are not ligated into place).
F. Synthesis of Disulfide-Labeled λ-DNA with Nineteen Nicks

Note: N.Bpu101 and its reaction buffer (#ER1681) was obtained from Fermentas.

F.1. In a Vial

1. Mix the following: 10 µL of 5 nM λ-DNA with the disulfide groups on the 3′ ends of opposite strands, 2 µL of N.Bpu101 (5U/µL) and 150 µL 1× reaction buffer.

2. Incubate vial at 37 °C for 1 h.

3. Pass solution from step 2 through a YM-100 filter to re-concentrate the sample after enzymatic digestion.

4. Dilute retentate according to quantitation assay to give a final concentration of 5 nM disulfide-labeled λ-DNA with nineteen nicks.

F.2. On the Device

1. To the disulfide-labeled λ-DNA aligned and attached to the Au electrodes, add 10 µL of a solution containing 1 µL of N.Bpu101 and 100 µL of 1× reaction buffer.

2. Place device in a sealed fluoroware container to prevent droplet evaporation and heat at 37 °C for 1 h.

3. After the allotted time, rinse device with deionized water and blow dry under a stream of nitrogen.
G. Repairing Nicks on the Device

1. Mix 2 µL of 10× ligase reaction buffer and 2 µL T4 DNA ligase (5 U/µL) and dilute this solution to 20 µL with deionized water.

2. Vortex solution to mix evenly.

3. Place a 10 µL droplet of this solution on the device and then place the device in a sealed fluoroware container. Put this container in the humidity chamber (both steps slow droplet evaporation).

4. Let droplet remain on device for at least 6 h at room temperature.

5. After the allotted time, rinse device with deionized water and dry device using a stream of nitrogen.
H. Synthesis of Single Stranded Disulfide-Labeled λ-DNA

Note: λ-DNA with disulfide groups on the 3’ and 5’ ends of the same strand was made single stranded using either a thermal/chemical reaction or an enzymatic reaction. A 10 nM concentration of double stranded DNA was used because half of the strands (those that are not labeled) are lost prior to measurement. Formamide was acquired from Amresco and λ-exonuclease with its reaction buffer (#LE035H) was obtained from Epicentre.

H.1. Denaturation

1. Dilute disulfide-labeled λ-DNA in formamide to give a final concentration of 10 nM.

2. Incubate vial at 95 °C for 5 min to thermally disrupt hydrogen bonding and immediately snap-cool on ice to prevent re-hybridization.

H.2. λ-Exonuclease

1. Mix 5 µL of 20 nM disulfide-labeled λ-DNA with 1 µL of λ exonuclease (10 U/µL) and 1 µL of 10× reaction buffer. Dilute this solution to a total volume of 10 µL with deionized water.

2. Incubate vial at 37 °C for 2 h to perform enzymatic digestion.

H.3. Single Stranding on a Device

Note: Both of the methods used to form single stranded DNA can also be performed on the device after alignment/attachment of the disulfide-labeled, double stranded λ-DNA.

1. Add the appropriate enzymes and reaction buffers to the device (with a total volume added of ~10 µL).

2. Place the device in a sealed container and heat/cool at the necessary temperatures.
I. Synthesis, Characterization, and Labeling of Short λ-DNA

I.1. Restriction Enzyme Digestion

Note: Bpl/I enzyme, reaction buffer, and S-adenosylmethionine (SAM) were obtained from Fermentas (#ER1311).

1. Mix 40 µL of 10 nM λ-DNA, 4 µL of Bpl/I enzyme (5 U/µL), 20 µL of 10× reaction buffer, 4 µL SAM, and 132 µL deionized water.

2. Incubate at 37 °C for 1 h to perform enzymatic digestion.

3. Inactivate enzyme by incubating at 65 °C for 20 min.

I.2. Slab Gel Electrophoresis

Note: 1× TBE buffer consists of 89 mM Tris-HCl, 89 mM boric acid, and 2 mM EDTA, at pH 8.3.

1. Add 1 g of agarose to 100 mL of 1× TBE to make a 1% w/v solution.

2. Heat the solution to boiling on a hot place to dissolve the agarose.

3. Let solution cool to 55 °C and then add 13 µL of 10 mg/mL ethidium bromide (EtBr) solution. Mix dye throughout solution.

4. Place comb into a gel plate and add half of the agarose solution.

5. Let the gel cool to room temperature.

6. Carefully remove the comb.

7. Place gel and holder in an electrophoresis system. Pour 300 mL of 1× TBE buffer (containing 40 µL of 10 mg/mL EtBr per 300 mL of buffer) into the buffer chambers so that the gel is just covered.

8. Mix 5 µL of gel loading dye with 5 µL of restriction-cut DNA or 1 kb sizing ladder. Heat samples at 65 °C for 2 min prior to loading. Pipet 10 µL of sample/dye or ladder/dye mixtures into wells.

9. Electrophorese the samples at 110 V for 45 min or until the dye has migrated past the center of the gel.

10. Image gel in the UV light box.
I.3. Disulfide Labeling

Note: An oligonucleotide complementary to the 5 base overhanging region of λ-DNA (introduced using Bp/I) was ordered from IDT with a 5’ C6 S-S modifier. This oligonucleotide was diluted to $2.5 \times 10^{-4}$ M with deionized water. λ2 was labeled with a 3’ C3 S-S modifier and diluted to a concentration of $1.4 \times 10^{-4}$ M.

Short λ: 5’ AAAGC 3’
λ2: 5’ GGGCGGCGACCT 3’

1. Phosphorylate λ2 at its 5’ end using T4 polynucleotide kinase: Take 3 μL of λ2 ($1.4 \times 10^{-4}$ M) and add 20 μL of 10× reaction buffer A, 2 μL ATP (0.1 mM), and 8 μL T4 polynucleotide kinase (10 U/μL). Dilute to a final volume of 200 μL with deionized water and incubate at 37 °C for 1 h.

2. Hybridize phosphorylated λ2 to λ-DNA by mixing the solution from step 1 with 20 μL of Bp/I cut λ-DNA (10 nM) (there is a 10³-fold excess of λ2). Heat solution to 75 °C and then cool slowly to room temperature by turning off the water bath or thermal cycler.

3. Ligate λ2 to λ-DNA by adding 40 μL of 10× ligation buffer and 4 μL of T4 DNA ligase (5 U/μL) to the solution from step 2. Vortex and spin down and then incubate at 22 °C for 12 h.

4. Pass solution from step 3 through a YM-100 filter to remove excess λ2.

5. Dilute λ-DNA/λ2 retentate from step 4 to 50 μL with deionized water and hybridize and ligate the short λ oligonucleotide to this molecule by adding 1.7 μL of the $2.5 \times 10^{-4}$ M solution and following steps 2 and 3.

6. Pass solution from step 5 through a YM-100 filter to remove excess short λ.

7. Dilute retentate according to quantitation assay to give a final concentration of 5 nM disulfide-labeled short λ-DNA (7460 bp).
J. DNA Quantitation

Note: Calf thymus DNA standard (#80622706) and the fluorescent dye, bisbenzimide (commonly known as Hoechst 33258, #80622687) were obtained from Amersham Biosciences. TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.4) and a 10× fluorescent assay buffer (100 mM Tris-HCl, 10 mM EDTA, 2 M NaCl, pH 7.4) were prepared for this procedure. A Perkin Elmer LS 45 Spectrometer was used for fluorescence measurements.

J.1. DNA Standard Calibration Curves

Note: A dye concentration of 0.1 µg/mL is adequate for the analysis of DNA at a final concentration up to ~500 ng. A dye concentration of 1 µg/mL will extend the assay’s range to 10 µg but will also limit the assay’s sensitivity at low concentrations.

1. Make a stock standard solution of calf thymus DNA by diluting with TE buffer to a concentration of 1 mg/mL.

2. Make a stock solution of bisbenzimide by diluting with deionized water to a concentration of 1 mg/mL (store in dark in the refrigerator).

3. Add the amounts of the reagents indicated in the two tables below. Mix thoroughly.

<table>
<thead>
<tr>
<th>Amount for 100 µg/mL calf thymus DNA solution</th>
<th>Amount for 10 µg/ml calf thymus DNA solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf thymus DNA (1 mg/mL)</td>
<td>100 µL</td>
</tr>
<tr>
<td>10× Fluorescent assay buffer</td>
<td>100 µL</td>
</tr>
<tr>
<td>dH2O</td>
<td>800 µL</td>
</tr>
<tr>
<td>Total volume</td>
<td>1 mL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amount for 1 µg/mL bisbenzimide solution</th>
<th>Amount for 0.1 µg/ml bisbenzimide solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>bisbenzimide (1 mg/mL)</td>
<td>30 µL</td>
</tr>
<tr>
<td>10× Fluorescent assay buffer</td>
<td>3 mL</td>
</tr>
<tr>
<td>dH2O</td>
<td>27 mL</td>
</tr>
<tr>
<td>Total volume</td>
<td>30 mL</td>
</tr>
</tbody>
</table>

4. Turn on fluorimeter and let it warm up. Set excitation wavelength to 360 nm and emission wavelength to 460 nm.
5. Use the following two tables to make two standard calibration curves (thoroughly mix each of the samples and cover in foil).

Table 3: Assay with 0.1 µg/ml bisbenzimide for DNA in the range of 10-500 ng/mL

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume of 10 µg/ml DNA standard</th>
<th>Volume of 100 µg/mL DNA standard</th>
<th>0.1 µg/mL bisbenzimide solution</th>
<th>Final amount of DNA in 2 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2 mL</td>
<td>Blank</td>
</tr>
<tr>
<td>2</td>
<td>2 µL</td>
<td>0</td>
<td>2 mL</td>
<td>20 ng</td>
</tr>
<tr>
<td>3</td>
<td>5 µL</td>
<td>0</td>
<td>2 mL</td>
<td>50 ng</td>
</tr>
<tr>
<td>4</td>
<td>10 µL</td>
<td>0</td>
<td>2 mL</td>
<td>100 ng</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>2 µL</td>
<td>2 mL</td>
<td>200 ng</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>5 µL</td>
<td>2 mL</td>
<td>500 ng</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>10 µL</td>
<td>2 mL</td>
<td>1000 ng</td>
</tr>
</tbody>
</table>

Table 4: Assay with 1 µg/ml bisbenzimide for DNA in the range of 100 ng/mL-5 µg/mL

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume of 100 µg/ml DNA standard</th>
<th>Volume of 1 mg/mL DNA standard</th>
<th>1 µg/mL bisbenzimide solution</th>
<th>Final amount of DNA in 2 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2 mL</td>
<td>Blank</td>
</tr>
<tr>
<td>2</td>
<td>2 µL</td>
<td>0</td>
<td>2 mL</td>
<td>200 ng</td>
</tr>
<tr>
<td>3</td>
<td>5 µL</td>
<td>0</td>
<td>2 mL</td>
<td>500 ng</td>
</tr>
<tr>
<td>4</td>
<td>10 µL</td>
<td>0</td>
<td>2 mL</td>
<td>1 µg</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>2 µL</td>
<td>2 mL</td>
<td>2 µg</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>5 µL</td>
<td>2 mL</td>
<td>5 µg</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>10 µL</td>
<td>2 mL</td>
<td>10 µg</td>
</tr>
</tbody>
</table>

6. Pipet 120 µL of the appropriate bisbenzimide assay solution into the microcuvette and place in the sample chamber.

7. Read the blank and DNA samples at 460 nm emission and at ambient temperature.

8. Thoroughly rinse and dry the cuvette between samples.

9. Repeat each sample measurement two additional times.

10. Prepare a calibration curve by plotting total DNA amount vs. fluorescence units (with the blank RFU value subtracted).
J.2. Measurement of the Concentration of an Unknown Sample Using 0.1 µg/mL Bisbenzimide

1. Prepare 0.1 µg/mL bisbenzimide solution in 1× fluorescent assay buffer by following Table 2 above.

2. Measure intensity at 460 nm for the bisbenzimide blank. Repeat 2 more times and take the average of the two measurements.

3. Add 2 µL of unknown DNA to 2 mL of 0.1 µg/mL bisbenzimide solution (following Table 3).

4. Read the intensity of the DNA sample at 460 nm. Repeat 2 more times and take the average of the three measurements.

5. Subtract the average blank reading from average unknown sample reading.

6. Using the value obtained in step 5 and the calibration curve equation obtained for the first linear range, calculate the amount (ng) and then the concentration (nM) of DNA present.

7. Multiply the value from step 6 by the normalization factor for λ-DNA (1.18), because this assay is sequence dependent (bisbenzimide binds preferentially to the minor groove of A/T sequences).
K. Synthesis, Characterization, and Labeling of Repetitive DNA

K.1. Enzymatic Blunt-Ending and Ligation

Note: Repetitive DNA sequences were ordered from IDT and diluted to 4×10^{-4} nM with deionized water. T4 polynucleotide kinase and its 10× reaction buffer (#EK0031), the Rapid DNA Ligation kit (#K1422), 50% (w/v) PEG 4000 (#EL0334), 100 mM ATP (#R0441), 2 mM dNTP mix (#R0241), and T4 DNA polymerase and its 5× reaction buffer (#EP0061) were obtained from Fermentas. The length of the starting materials were poly(AT): 30 bases, poly(A) and poly(T): 40 bases, and poly(G) and poly(C): 20 bases. The following procedure is for the ligation of poly(A)/poly(T) however, the same procedure can be utilized for any of the repetitive complementary strands.

1. Phosphorylate poly(A) at its 5′ end using T4 polynucleotide kinase: Take 1 µL of poly(A) (4×10^{-3} M) and add 8 µL of 10× reaction buffer A, 0.3 µL ATP (100 mM), and 2 µL T4 polynucleotide kinase (10 U/µL). Dilute to a final volume of 80 µL with deionized water and incubate at 37 °C for 1 h.

2. Repeat step 1 with poly(T).

3. Hybridize poly(A) to poly(T): Mix the solutions resulting from steps 1 and 2 together and heat at 90 °C and then cool slowly to room temperature by turning off the water bath or thermal cycler.

4. Blunt-end the hybridized product: To the solution from step 3, add 4 µL of T4 DNA polymerase, 32 µL 5× reaction buffer, and 4 µL 2 mM dNTP mix. Incubate at 11 °C for 40 min. Heat inactivate enzyme at 70 °C for 10 min.

5. Ligate the blunt-ended material: To 10 µL of the mixture from step 4 add 4 µL of 5× rapid ligation buffer (from Rapid Ligation kit), 1 µL T4 DNA ligase (from Rapid Ligation kit), 4 µL PEG 4000, and 1 µL deionized water. Incubate at 22 °C for 1 h.

K.2. Capillary Electrophoresis Using 2% HEC in TBE

Note: The sieving matrix consisted of 2% HEC (MW 250,000) and with this polymer solution a 1× TBE run buffer was utilized. Detection was accomplished using either TO-PRO-3 iodide or YO-PRO-1 iodide and a 635 nm diode laser or a 488 nm Ar ion laser, respectively. Separations were performed on a P/ACE 2050 or a Beckman MDQ at room temperature.
1. Slowly add enough HEC powder to TBE buffer while mixing with a magnetic stir bar to make a 2% (w/v) solution. Pause periodically to prevent large clumps of HEC from forming.

2. Cover the solution and allow it to stir overnight to ensure that all of the HEC is dissolved.

3. Filter the solution using a 5 \( \mu \)m Gelman Acrodisc syringe tip filter and store at 4 °C.

4. Add either 0.8 µL of 1 mM TO-PRO-3 or 1 µL 1 mM YO-PRO-1 per 5 mL of both the sieving matrix and run buffer solutions. Vortex solutions to mix evenly and sonicate for 1 min to remove any bubbles that have formed.

5. Rinse a 37 cm long fluorocarbon-coated capillary (50 \( \mu \)m internal diameter) with run buffer (TBE) for 2 min at high pressure.

6. Fill the capillary with sieving matrix by rinsing for 6 min at high pressure.

7. Electrokinetically inject sample for 5 s at 10 kV.

8. Use a run voltage of 8 kV (reverse polarity) and TBE buffer for DNA separation.

9. Between runs rinse the capillary with deionized water for 2 min at high pressure.

**K.3. Capillary Electrophoresis Using 5.5% PVP in TBE**

Note: The sieving matrix consisted of 5.5% PVP (MW 1,000,000) and with this polymer solution a 1× TBE run buffer was utilized. Detection was accomplished by adding YO-PRO-1 iodide to either the separation system (run buffers and sieving matrix) or directly to the DNA sample. Separations were performed on a P/ACE 2050 system at room temperature with a 488 nm Ar ion laser.

1. Add enough PVP powder to TBE buffer to make a 5.5% (w/v) solution.

2. Cover solution and mix evenly with a magnetic stirrer for 1 h.

3. If dye is to be added to the run buffers and sieving matrix, 1 µL of 1 mM YO-PRO-1 is added per 5 mL of solution. Vortex solutions to mix evenly and sonicate for 1 min to remove any bubbles that have formed.

4. If dye is added to the sample, mix 0.2 µL of 1 mM YO-PRO-1 with 1 µL of the ligation product or 0.5 µL of the sizing standard, pBR 322/Hae III digest (148
µg/mL), and 100 µL of deionized water. Vortex solution to mix evenly and let sample sit at ambient conditions for 10 min.

5. Fill a 37 cm long uncoated capillary (50 µm internal diameter) with sieving matrix by rinsing for 6 min with high pressure.

6. Electrokinetically inject the sample for 2 s at 2 kV.

7. Use a run voltage of 5 kV (reverse polarity) and TBE buffer for DNA separation.

8. Between runs rinse the capillary with deionized water for 2 min at high pressure and then methanol for 2 min at high pressure.

K.4. 5’-Thiol Labeling

Note: The 5’ EndTag™ Nucleic Acid Labeling System (#MB-9001) was obtained from Vector Laboratories.

1. Reconstitute ATPγS in 10 µL deionized water.

2. To remove the 5’ phosphates on the DNA and replace them with 5’ hydroxyl groups: Mix 1 µL universal reaction buffer, 1 µL alkaline phosphatase and 8 µL of 1 µM ligated poly(A)/poly(T) mixture or any repetitive DNA mixture. Incubate at 37 °C for 30 min.

3. To label the dephosphorylated DNA: Combine and mix 2 µL of universal reaction buffer, 2 µL T4 polynucleotide kinase, 1 µL ATPγS, entire reaction mixture from step 1, and 5 µL of deionized water. Incubate at 37 °C for 30 min.