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UNIVERSITY OF CALIFORNIA
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Nanotechnology Applications in Self-Assembly and DNA Computing

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

Doctor of Philosophy

in

Electrical Engineering

by

Hayri Engin Akin

June 2011

Dissertation Committee:

Dr. Mihrimah Ozkan, Chairperson

Dr. Cengiz S. Ozkan

Dr. Roger Lake

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The Dissertation of Hayri Engin Akin is approved:

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University of California, Riverside

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DEDICATION

I dedicate my work to my family, Dr. Tayfur Akin, Z. Esin Akin and Ipek Akin. All credit is yours.

ABSTRACT OF THE DISSERTATION

Nanotechnology Applications in Self-Assembly and DNA Computing

by

Hayri Engin Akin

Doctor of Philosophy, Graduate Program in Electrical Engineering

University of California, Riverside, June 2011

Dr. Mihrimah Ozkan, Chairperson

Nanotechnology spans and merges very diverse areas from device physics to molecular self-assembly, from development of new materials with nanoscale dimensions to manipulating existing materials on atomic scale. DNA nanotechnology is the field of nanotechnology that uses the unique structure and properties of DNA as a structural material or computational medium. DNA nanotechnology has various applications but mostly is used in DNA computing and molecular self-assembly. In this dissertation these two areas are investigated. Under DNA-based nanofabrication, construction of functional materials as building blocks for nanoelectronics and optimal DNA sequence design for assembly are researched. Conjugation of carbon nanotubes as well as end-to-end assembly of nanowires with DNA is demonstrated, electrical measurements are investigated, and the process is carried on an electronic microarray platform. This electronic microarray platform is adopted to perform DNA computing, which is the biggest accomplishment of this dissertation. The information present in an image is encoded through various DNA strands and decoded on a CMOS-enabled platform to recreate the original image. Satisfiability problems were solved via 2 different methods. It is notable that the proposed approach eliminated the need for PCR and enzymes, resulting

in a decreased error rates and cost. Overall, this technique shows significant advantages over previous experimental techniques such as short operating time, reusable surface and simple experimental steps. Finally, microelectronics and molecular biology techniques are integrated for showing the feasibility of Hopfield neural network using DNA molecules. Six-dimensional Hopfield associative memory storing various memories is demonstrated as an archetype neural network using DNA. The results are read on an electronic microarray platform, which opens the semiconductor processing knowledge for fast and accurate hybridization rates. The research undertaken under the umbrella of this dissertation is expected to have broad implications for next-generation functional materials such as nanoscale building blocks. The proposed surface-based DNA computation approach will bring the hybrid concept of silicon-compatible DNA computing to realization. Integration of a CMOS platform and DNA for a completely non-biological purpose has the potential to greatly affect the future applications.

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1. Introduction

Nanotechnology is a diverse and revolutionary area of research with potential applications that can profoundly impact society. Nanotechnology involves device physics, molecular self-assembly, development and manipulating new and existing materials at the atomic scale. Therefore, it is believed that nanotechnology will be at the forefront of global technology development¹⁻². The benefits of nanotech will go beyond electronic applications; with smaller and smarter circuits, it will span into biomedical, renewable energy and pharmaceutical applications to name a few³⁻⁴.

If we are planning to continue to increase the complexity and computational power of electronic devices, we need to address the physical and economical limitations of top-down approaches in scaling-down the current CMOS technology. To reach smaller, faster, cheaper electronic and computational units, innovations and developments of new device structures, materials and concepts are needed⁵. In this thesis nanotechnology applications with carbon nanotubes (CNTs) and nanowires (NWs) are investigated where DNA is utilized as the smart linkage to assemble the desired nanostructures. Moreover, DNA is used as the computation medium and DNA computing is carried on an electronic microarray platform with various projects. In the interests of overall clarity of the dissertation, we first introduce the key components: CNTs, NWs, DNA, electronic microarray platform and DNA computing.

Carbon nanotubes (CNTs)

Since the discovery of carbon nanotubes⁶ in 1991 there has been a great interest in the synthesis, characterization and fabrication of one-dimensional (1D) structures. Carbon nanotubes (CNTs) emerged as potential candidates for next-generation electronics due to their extraordinary strength and unique electrical properties as well as their thermal conduction efficiency⁷. The applications proposed with CNTs are not limited to electronics. Optics, materials science and architectural fields can also benefit from these novel nanostructures^{7, 11, 12}.

CNTs can be categorized as single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs). SWNTs can be thought of as seamlessly wrapping a single graphite layer into a cylindrical tube. MWNTs, on the other hand, have an array of such nanotubes and can be considered multiple rolled layers of graphite. SWNT diameter is approximately 1 nm, and its length can go up to centimeters^{1, 8}. SWNTs may be semiconducting or metallic depending on the direction in which the graphite sheet is rolled to make the nanotube. The direction in the graphite sheet and nanotube diameter can be described with a pair of indices (n,m). This is called chiral vector, and it defines the type of the nanotube. When (n=m) it is called armchair, (n=0, m=0) gives zigzag, and other combinations are named chiral^{1, 8}. All armchair SWNTs are metallic tubes⁸.

Nanotubes with $n - m = 3k$ where k is a nonzero integer are semiconducting with very small band gap. All other tubes have a band gap inversely depending on the nanotube diameter^{1, 8-10}. Electrical properties of MWNTs are considered zero band gap materials.

Due to their nearly 1D electronic structures, ballistic electron transport occurs in SWNTs and MWNTs¹¹⁻¹⁶. Phonons propagate easily along the nanotubes. Measured room temperature thermal conductivity for individual nanotubes are around 3000 W/mK, which is greater than natural diamond and basal plane of graphite (both 2000W/mK¹⁷). CNTs can be synthesized by various methods such as CVD, High Pressure CO (HiPCO) disproportionation process, arc method or laser ablation method¹⁸⁻²².

In theory, many applications can be realized while using CNTs. One example is CNT-based supercapacitors where a CNT's electrochemically functionalized surface and high electron conduction are combined²³. Nanometer separation in CNT structure enables large amount of charge injection when only a few volts are applied. This idea can be used for high-power applications such as batteries and hybrid electric vehicles^{23,24}. Other areas of proposed CNT applications are textiles (wear-resistant, waterproof clothes²⁵), construction²⁶ (bridges and building where CNT increases the tensile strength of concrete), artificial muscles²⁷, nanotweezers, and chemical and biological sensors^{28,29}.

For electrical engineers, CNT research focuses on the promise of next-generation nano-electronic circuits³⁰. The semiconductor industry is continuing to shrink transistor dimensions, but materials start to play a significant role as the dimensional scaling decreases³¹. Electronic circuits cannot continue indefinitely to shrink by orders of magnitude and provide increased computational power, so different materials, architectures and assemblies are continuously developed⁵. Intel brought a fundamental change in the structure of the transistor since 1960s and introduced high-k metal gate

transistors³². These recent advances reveal that nanotubes or nanowires can be one of the candidates for future downsizing circuit elements. For example, current-induced electron migration causes conventional metal wire interconnects to fail when the wire dimension is reduced in the nano dimensions. The covalently bonded CNTs can be a solution to these limitations because of their ballistic transport³³. It is expected that nanotube electronics will be realized in about a decade³⁴⁻³⁶. In reaching that goal many technical challenges must be overcome. Large scale alignment at desired locations is one of the most important challenges.

Nanowires (NWs)

Future semiconductor technology requires alternative materials and technologies in addition to smaller dimensions⁵. Among many nanoscale materials researched, nanowires, nanorods and nanotubes are promising materials for many novel applications from chemical and biological sensors to optical and electronic devices³⁷⁻³⁹. Compared with bulk materials, nanoscale materials, with their large surface area and possible quantum-confinement effects, exhibit distinct electronic, optical, chemical and thermal properties⁴⁰. One-dimensional nanostructures with well defined dimension, composition and crystallinity represent a new class of intriguing system for investigating structure property relationships and related applications. Many reports reveal theoretically as well as experimentally that NWs have various different properties than their bulk counterparts due to their structure⁴¹⁻⁴³. NWs have size-dependent mechanical properties, increased luminescence efficiency, reduced threshold for lasing and enhanced electromechanical

response^{40, 44-48}. NWs have been used in research as building blocks in electronics, optics and mechanics such as from using as a master in nanoimprinting to masking in dry etching⁴⁹.

There are various methods for NW or nanorod fabrication, but we can summarize the main methods in four categories.

1. Vapor-Liquid-Solid (VLS) Process⁵⁰:

The steps of VLS process are the formation of a small liquid droplet, alloy formation with the substrate, nucleation and growth of the NW. Small metal particles with a diameter below 100 nm are introduced to the surface of the substrate. They initiate the NW growth^{50, 51}. These substrates are placed in a reaction tube or chamber such a CVD chamber and are heated until the metal particles turn into liquid droplets. The temperature is in the range of 350C to 1100C depending on the binary phase diagram between the metal and the chosen target material. Next, the gas containing the growth material is introduced to the chamber. The liquid droplet has a much larger sticking coefficient than the surface of the substrate, so the precursor atoms prefer to deposit on the surface of the liquid and form the alloy⁴⁹. Continuous flow of the gas causes the supersaturation of the liquid droplets and crystal growth occurs at the solid-liquid interface by precipitation. The diameter of the NW grown in this way is determined by the size of the droplet⁵². The disadvantages of this method are high temperature, costly apparatus, difficulties in obtaining heterostructure NWs and problems in controlling the NW growth direction^{40, 49}.

2. Vapor-Solid (VS) process:

In this method NWs are grown without the metal catalysts. Source material is evaporated near its melting point and deposited at lower temperatures⁴⁹. This self-organizing process is similar to VLS process, but here the gas atoms play the role of the metal particles as catalyst. The NW diameter is controlled by the evaporation and collection temperatures as well as vapor pressure. Various semiconducting NWs can be grown with this method⁵³.

3. Solution growth:

Mainly combining chemicals, temperature, solvent and solute properties of reactions, desired materials are precipitated in its 1D nanoscale form. The main advantages of this method are its low-temperature operation, low cost and availability of various substrates. Some of the disadvantages of this method are control of the structural features as well as area density. Also, these NWs have smaller aspect ratio⁵⁴.

4. Electrochemical deposition (ECD):

NWs can be grown by electrochemical deposition inside templates such as porous or nano-channel glass. The template is attached to the cathode and introduced to the deposition solution inside an electrochemical cell. The anode is placed in the deposition solution parallel, and electric field is applied. Cations diffuse and reduce at the cathode, resulting in the growth of NWs inside the pores of the template⁵⁵. The advantage of this method is the large-scale, low-cost fabrication of supported NWs. When combined with lithography techniques, this method can have potential applications in nanotechnology. The disadvantage of this method is quality of the nanowires produced, which are mainly

not crystalline⁴⁰. One of the most important factors in the NW synthesis is the control of composition, size and crystallinity⁴⁹. ECD method is very suitable for our research for testing our assembly ideas with DNA. In chapter 2, various NWs are fabricated via template directed electrochemical deposition (ECD). The architecture of the porous template defines the shape, direction and size of the wire. The idea we wanted to test is not the NW fabrication but testing whether CNT or NW as a circuit element can be assembled via DNA. The advantages of the template-directed growth technique are low cost, high throughput, high volume and ease of production. The nanowires can be released from the template matrix by chemical dissolution of the template. The diameters of NWs range from a few nanometers (nm) to few hundreds of nm, and the length vary over even greater range from few nm to many microns. Also, multi-component NWs can be grown simply by changing the deposition solution composition. Elemental, semiconducting and multi-component NWs can be produced by this method. We have explored in the lab the fabrication of metallic and semiconducting materials such as Au, Ag, Ni, InSb^{40, 49, 55}.

DNA

Deoxyribonucleic acid (DNA) is a nucleic acid containing the genetic information used in the development and functions of organisms⁵⁶. DNA is taught as the blueprint of life, because it has the instructions necessary to construct other components of cells, such as proteins and RNA. The DNA segments carrying this genetic information are called genes, but other DNA sequences have structural purposes, or are involved in regulating the use

of this genetic information⁵⁶⁻⁵⁸. DNA is a long polymer consisting of simple units called nucleotides and has a backbone made of sugars and phosphate groups joined by ester bonds. Each sugar has one of four types of molecules called bases (adenine (A), cytosine (C), guanine (G) and thymine (T)). The sequence of these four bases along the backbone encodes information^{56, 57}.

DNA chain is 2.2 to 2.6 nanometers wide, and one nucleotide unit is 0.33 nm long⁵⁸. Although each individual unit is very small, DNA polymers can be very long chains with millions of nucleotides. For example, human chromosome number 1 is approximately 220 million base pairs long⁵⁹. In organisms, DNA exists as a pair of molecules, double helix⁵⁹⁻⁶¹. The backbone of the DNA strand consists of alternating phosphate and sugar groups^{56, 63}. A phosphodiester bond is a group of strong covalent bonds between the phosphorus atom in a phosphate group and two other molecules over two ester bonds; together, they make up the backbone of the strands of DNA⁶⁰⁻⁶². Because of these asymmetric bonds, DNA strand has a direction. In a double helix the direction of the nucleotides in one strand is opposite to their direction in the other strand. The asymmetric ends of DNA strands are referred to as the 5' (five prime) and 3' (three prime) ends, the 5' end terminates with a phosphate group and the 3' end terminates with a hydroxyl group⁵⁶⁻⁵⁸. DNA double helix is stabilized by hydrogen bonds between the bases (A, C, G, T) of two strands. Each type of base on a strand makes a bond with only one type of base on the other strand: A bonds only to T, and C bonds only to G^{56, 59}. This bonding of two nucleotides across the double helix is called a base pair. The double helix is also stabilized by the hydrophobic effect and pi stacking, which are not influenced by the

sequence of the DNA^{64-67, 70}. The hydrogen bonds in complementary base pairs are not covalent. They can be broken and rejoined easily. The two strands of DNA in a double helix can be pulled apart either by a mechanical force or high temperature⁶⁸. As a result, all the information in the DNA helix is duplicated on each strand, which is vital in DNA replication. This reversible and specific interaction between complementary base pairs is critical for all the functions of DNA in organisms⁵⁶⁻⁶⁰. Base pairs form different numbers of hydrogen bonds. A and T forms two hydrogen bonds, and GC forms three hydrogen bonds⁵⁶⁻⁵⁸. Therefore, the GC base pair is stronger than the AT base pair. As a result, both the percentage of GC base pairs and the overall length of a DNA double helix determine the strength between the two strands of DNA. Long DNA helices with a high GC content are strong interacting strands, while short helices with high AT content are weaker strands⁶⁸⁻⁷⁰. The strength of the strands can be determined by finding the temperature to break the hydrogen bonds, which is a very approximate definition of the melting temperature (T_m) of the sequence. The strands separate in the solution and act as independent molecules when these hydrogen bonds are broken⁶⁸⁻⁷¹. Determining the melting temperature of an oligo is vital for many applications from PCR to mutagenesis, hybridization and sequencing⁷¹⁻⁷⁵.

Electronic Microarray

DNA microarray is a molecular biology technology used in biology and in medicine. It is an array of thousands of microscopic spots of oligonucleotides, where each spot contains picomoles of specific DNA sequence^{76, 77}. These sequences can be a short section of a

gene, another DNA sequence used as probes to hybridize a complementary DNA or a complementary RNA sample under high-stringency conditions⁷⁷⁻⁸⁰. Hybridization is usually detected by fluorescence based methods. Fluorophore labeled target determines the relative amount of nucleic acid sequences in the target. Microarray technology makes it possible to look at many genes at once and to determine which are expressed in a particular cell. Thousands of individual genes can be spotted on a single square inch slide due to base pairing. After washing away all of the unstuck sequences, we can look at the microarray under a microscope and see which sequences remain stuck to the DNA spots⁸⁰⁻⁸⁴. Every sequence at each spot is known and DNA only sticks to the complementary one. This technology is used especially to learn which genes are turned on or off in diseased versus healthy cells.

The success of microarrays not only depends on the chemistry used for the immobilization of oligonucleotides but also depends on the good accessibility and functionality of the surface-bound oligonucleotides, density of attachment, thermal stability of the array under experimental conditions and reproducibility of attachment chemistry⁷⁶⁻⁸⁰. Basically, there are two approaches for the fabrication of DNA arrays. At the first approach, oligonucleotide probes are directly synthesized on the surface at pre-selected positions following conventional photolithographic techniques⁷⁸⁻⁸³. This methodology is by far the most efficient method for the construction of high-density oligonucleotide arrays, however, it has practical limitations in terms of flexibility and affordability⁸⁴⁻⁹⁰. The second method, called the deposition method, where pre-fabricated nucleic acids are covalently or non-covalently immobilized on solid surfaces, offers an

excellent flexibility⁹¹⁻⁹⁴. This latter approach has thus become the most widely used method for creating low- to medium-density DNA microarrays.

Since 2007 in Professor Mihri Ozkan's Lab in UC Riverside, we have the NanoChip 400 microarray platform. NanoChip (NC) 400 System is an automated platform for molecular testing designed by Nanogen Inc. This system has various advantages relative to the above mentioned array platforms due to its microelectronic nature⁹¹⁻⁹⁶. I utilized the greater flexibility of this microelectronic chip in my research, which will be explained further in the dissertation. Nanochip[®] technology was bought from former Nanogen (San Diego, CA) by Gamida Group in 2009. Various applications and assay kits were developed for Genetic Carrier Screening (such as Cystic Fibrosis, Tay Sachs, Ashkenazi panel, Gaucher, Connexin and more).

Before proceeding forward, I would like to introduce briefly the system that I used for my experiments. NC 400 is an electronic microarray technology for manipulation, concentration and hybridization of biomolecules on the chip array⁹⁷ (Figure 1.1). Each test site on the array is connected to an electrode.

Targeted molecules concentrate at the array site, where they can be bound chemically or hybridized to a DNA probe. Fluorescent signal is obtained from the reporter probes hybridized to the target DNA, and signal proportional to the concentration of DNA is measured⁹⁶. Most biological molecules have a natural positive or negative charge.

Therefore, when biological molecules are exposed to an electric field, they move to the electrode with opposite charge (Figure 1.1)⁹⁷. Here, DNA molecules due to their negative

charged backbone move to electrodes with a positive potential. Current or voltage is applied to a test site/pad via individual electrode activation to facilitate the rapid and controlled transport of charged molecules to any test sites⁹⁶⁻⁹⁸.

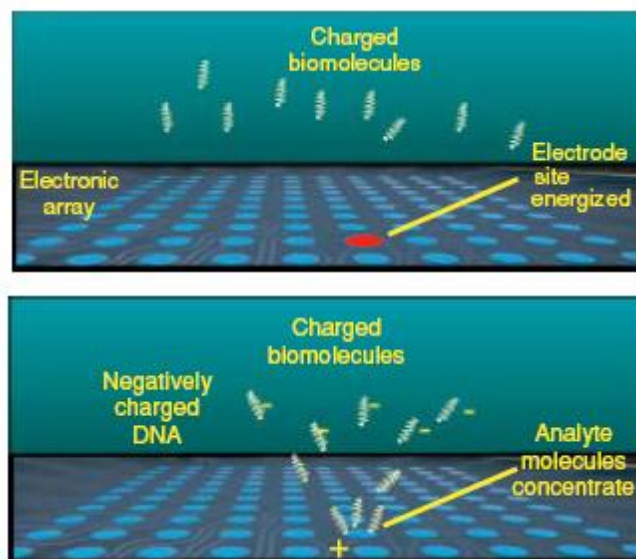


Figure 1.1: Nanochip microarray technology uses electronic addressing of charged biomolecules on the electrode array to separate and concentrate targets. Negatively charged DNA targets and molecular probes (top) are moved to a particular site by energizing the electrodes at a reverse potential (bottom)⁹⁷.

Additional advantages of electrically facilitated transport due to the ability to produce reconfigurable electric fields on the microarray surface are the ability to carry out site selective DNA addressing and hybridization, the ability to significantly increase DNA hybridization rate by concentration of target at the test sites, and the ability to use electronic stringency to improve hybridization specificity⁹⁶⁻⁹⁹.

NanoChip 400 is an automated system including electronic microarray and workstation (Figure 1.2)⁹⁷. Electronic microarrays consist of an array of 400 platinum micro electrodes fabricated on silicon using standard photolithography and deposition

processes. Each electrode is 80 μm in diameter with 150 μm center-to-center space⁹⁷. The base of the array is silicon over which an insulating layer of silicon dioxide is applied. Platinum is deposited and electrodes are formed with accompanying electrical traces. On the surface of the array, there is a 10 μm thick hydrogel permeation layer which contains streptavidin⁹⁶. This permeation layer serves two main functions. First, it protects the sensitive analytes from the adverse electrochemical effects at the platinum electrode surface during activation of a pad⁹⁷. These electrochemical products include the generation of hydrogen ions (H^+) and oxygen at the positively biased (anode) microelectrodes and hydroxyl ions (OH^-) and hydrogen at the negatively biased electrodes (cathode), as well as various free radical entities⁹⁶⁻⁹⁸. Secondly, the permeation layer provides the attachment medium of biotinylated molecules through biotin and streptavidin binding^{96, 97}. NanoChip 400 array is assembled into a cartridge (Figure 1.2) by ultrasonically welding two molded polymethyl methacrylate (PMMA) cartridge bodies which contains fluidic channels, inlet and outlet ports⁹⁷. The cartridge eliminates sample evaporation, prevents contamination and provides a fluidic interface to the NanoChip Workstation⁹⁷. The NanoChip electronic microarrays are operated through an automated NanoChip Workstation (Figure 1.2). The system in addition to cartridges has three major subsystems. Inside the NanoChip 400 instrument, there are loader and reader units. Loader is for transferring samples, solutions and buffers on to NanoChip Cartridges and the NanoChip Reader includes a highly sensitive, laser-based fluorescence scanner for detection of assay results. As seen in Figure 1.2 these functions are operated with a

computer, which automates import, analysis and export of sample information making data analysis simple.

The NanoChip® 400 System

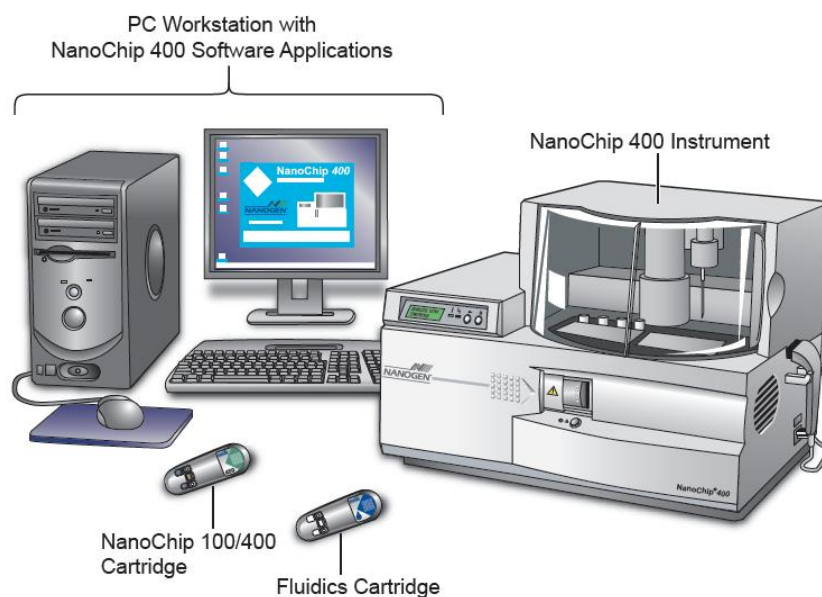


Figure 1.2: The NanoChip 400 System. The NanoChip 400 System consists of the following main components: NanoChip 400 Instrument, NanoChip 100/400 Cartridge, Fluidics Cartridge, NanoChip 400 Software, PC Workstation⁹⁷.

These features of NanoChip electronic microarrays provide various advantages over other passive microarrays⁹⁶⁻⁹⁹. Electronic microarray concentrates oligos at the specific array 1000 times more than the diffusion-dependent passive hybridization arrays in a much faster time frame (10-100 seconds versus 2-4 hours in passive hybridization)⁹⁷. Electronic addressing allows users to customize arrays⁹⁷⁻⁹⁹. Electronic stringency, in combination with thermal control, enables researchers to remove unbound and nonspecifically-bound DNA quickly and easily after hybridization at the microarrays, achieving rapid determination of single base mismatch mutations in DNA hybrids^{97, 98}. The ability to

electronically control individual test sites permits biochemically unrelated molecules to be used simultaneously on the same microchip. In contrast, sites on a conventional DNA array cannot be controlled separately, and all process steps must be performed on an entire array⁹⁶⁻⁹⁹. This increased versatility over conventional methods can be used in applications from biological warfare to infectious disease detection⁹⁷⁻⁹⁹. In this dissertation this novel instrument is utilized for the first time for DNA nanoelectronics and DNA computing research.

DNA Computing

DNA computing is a method of parallel computation which uses the selectivity of DNA denaturization to sift through a large space of combinations that would be possible starting with many different strands of DNA¹⁰⁰. Molecular computing or more specifically DNA computing took off in 1994, when L.M. Adleman in his paper “Molecular Computation of Solutions to Combinatorial Problems” used DNA to solve an example of the Hamiltonian Path Problem (HPP), experimentally demonstrating the use of DNA as a computational system¹⁰⁰. Adleman demonstrated computation at the molecular level with DNA molecules and biological reactions. In this pioneering paper, a small graph is encoded in DNA molecules and solved by using molecular biology¹⁰⁰. In order to appreciate Adleman’s study, HPP and its complexity should be understood. HPP is a special case of the traveling salesman problem, where a directed tour that starts at a given city, ends at a given city, and visits every other city exactly once for a given set of cities and directed paths¹⁰¹⁻¹⁰⁴. As the number of cities is increased, the computational

complexity of such problem increases exponentially. In Adleman's paper, the cities and roads are represented by vertices and edges, respectively. A graph with designated vertices v_{in} and v_{out} is said to have a Hamiltonian path if and only if there is a path consisting of "one way" edges that begins at v_{in} , ends at v_{out} , and visits every other vertex once and only once¹⁰⁰. Although there are algorithms for choosing whether a randomly formed graph for given vertices has a Hamiltonian path or not, it is not polynomial time efficient, because the HPP has been proven to be nondeterministic polynomial complete (NP-complete)^{105, 106}. So, these algorithms require huge amount of computer time to provide a decision. However, Adleman's algorithm simplifies the problem and makes it solvable via DNA. In his algorithm, the solutions were filtered and eliminated by various steps, and only those solutions meeting all the criteria to be a Hamiltonian path were left. The solution of the problem Adleman solved via DNA can be easily detected by visual inspection. However, it took 7 days of lab work to solve the same problem using DNA molecules¹⁰⁰. Although this experiment was slow, it was still a big step that demonstrated various merits of DNA computing. We can summarize here these important features of DNA computing: 1) DNA computing provides speed due to its parallel processing. When we consider an operation in molecular biology such as ligation of DNA molecules, the number of operations per second can be much bigger than supercomputers such as on the order of 10^{12} operations per second. 2) DNA computing is remarkably energy efficient. As Adleman calculated with 1 Joule, 2×10^{19} ligation operations can be carried. 3) Information can be stored in DNA theoretically up to 1 bit per cubic nanometer. This massive parallelism introduces the possibility that DNA computation may help to solve

certain problems more conveniently than could be done using an ordinary silicon computer¹⁰⁰⁻¹⁰⁵. An example would be a computation involving inputs that are already encoded in concentrations of various DNA strands, for example from a genomic library. In addition to this massive parallelism inherent in the biological operations, DNA computing also offers extremely dense information storage capacity and energy efficiency. Therefore, much work is being done to determine if this potential can be realized in practical use to solve real computational problems that cannot be solved using conventional methods.

After Adleman, in 1995, Lipton proposed a series of DNA experiments to solve the satisfaction (SAT) problem, another famous NP-complete problem¹⁰⁷, and showing that DNA computing can offer solutions to other NP-complete problems. A SAT problem is a decision problem written in Boolean expression¹⁰⁷. The goal in a SAT problem is to determine appropriate assignments of a set of Boolean variables with values of either “true” or “false” such that the output of the whole Boolean formula is true. In general, a SAT problem is a formula (such as $C_1 \wedge C_2 \wedge \dots \wedge C_m$) consisting of m clauses (C_i where $i=1, 2, \dots, m$) all of which are connected by “AND” ($\wedge$) operation. A clause C_i is of the form $v_1 \vee v_2 \vee \dots \vee v_k$ where each v_j ($j=1, 2, \dots, k$) is a variable or its negation connected by “OR” ($\vee$) operation¹⁰⁷. The importance of this finding as Lipton stated is that any Boolean expression may be put into normal form as a sum of products or a product of sums. Therefore the SAT problem as presented above is completely general¹⁰⁷.

After Lipton's proposal, various SAT problems were solved by several groups using different DNA computation techniques¹⁰⁷⁻¹¹⁰. Braich et. al. solved a 20-variable instance of this NP complete SAT problem – the largest yet solved computational problem by non-electronic methods¹¹¹. Solving SAT problems with DNA became the benchmark in DNA computing for testing the performance of the model.

In 2000, Liu et al. solved SAT problem using a surface-based DNA computation approach¹¹². Initial experiments in DNA computation¹⁰⁰⁻¹⁰⁴ used chemical manipulations carried out in test tubes. This approach required magnetic beads to remove DNA strands from the solution, which are error prone and not easy to scale up for larger problems even if we just consider the different types of beads required for a particular mathematical problem¹¹³. Surface-based DNA computation solves these problems because after immobilizing the DNA on a solid support, it becomes simpler to analyze DNA between the various steps of the computation¹¹². Next challenges for DNA Computing to overcome are improvements in control and reliability as well as reduction in cost and error. At that point, surface-based methods offer advantages that can make DNA Computing a candidate for commercialization and applications to difficult computational problems. In this dissertation, SAT problems are solved with electronic microarrays to show as a proof of concept that surface-based methods with semiconductor manufacturing techniques can be combined in DNA Computing for future commercialization and applications.

Next, I would like to give a little information about artificial neural networks related to our studies in collaboration with Professor Allen Mills. Our brains contain about 100 billion units called neurons¹¹⁴. Each neuron is connected to thousands of other neurons and communicates with them using electrochemical signals¹¹⁴⁻¹¹⁶. Neurons continuously receive input signals, sum them up in some way, and fire an output signal if the end result is greater than some threshold value¹¹⁷. The neural network is man's crude way of trying to simulate the brain electronically¹¹⁷⁻¹²⁰. A neural network is a set of computing elements. These elements can be connected in such a way as to store information, perform specific mathematical operations, or find patterns¹¹⁸⁻¹²⁰. Information processing capabilities of ANNs and their ability to learn from examples make them efficient problem solving models. Hopfield Neural Network (HNN) is suited to memory recall¹²⁰. A HNN stores information by presenting data patterns to be stored one by one to the network. This way the network learns from examples. Once these "memories" are stored, it is possible to recall them by presenting a piece of information^{119, 120}.

To create fault-tolerant computing algorithms with DNA, Mills, Platzman, Yurke (MYP) model HNN is researched in this dissertation in collaboration with Professor Mills¹²¹⁻¹²³. MYP network is a type of neural network in which DNA is used as the working substance, where HNN is implemented¹²¹⁻¹²³. Axons and neurons are replaced by DNA molecular reactions and matrix operations are performed on the network. Here, information is represented with sets of DNA sequences. Memory matrix elements are represented by sets of ligated oligomers from the memory vectors as an extension of

Oliver's matrix algebra¹²⁴, where Oliver described a method for calculating the product of matrices using DNA.

Research Focus

Top-down methods using lithography are getting more complicated and challenging with further miniaturization. To realize the nanoscale electronics, the development of bottom-up methods such as molecular self-assembly are required. One of the major goals of nanotechnology is coupling the self-assembly of molecular nanostructures with conventional microfabrication. A marriage of the bottom-up and top-down fabrication methods can enable us to register individual molecular nanostructures, to electronically address them, and to integrate them into functional devices. From this perspective, inorganic nano materials and DNA are the attractive building blocks for the next generation structures to build smaller, faster and more sophisticated devices. The idea behind this dissertation is researching novel bottom-up fabrication techniques like self-assembly and outside the box concepts with great potential like DNA computing. I tried to integrate these concepts with the tools provided by top-down approach. I truly believe these ideas can contribute to the development and realization of nanotechnology.

After this brief introduction, I hope the reader is more familiar with the materials we employed in the research and can continue to reading this dissertation research easily. Following the introduction chapter 1, this dissertation is divided into 4 chapters summarizing my research activities. Chapter 2 presents my efforts of functionalizing CNTs and NWs with DNA, manipulating them with an electronic microarray and

discusses the smart assembly method we propose. Chapter 3 presents my initial efforts of using electronic microarray technology to encode and decode information present in an image. In this chapter user defined information can be stored and recalled via DNA hybridization on an electronic microarray platform. In Chapter 4, I present an algorithm for solving SAT problems on the electronic microarray. In this study, microelectronics and molecular biology techniques have been integrated for accelerated transport of DNA molecules. This approach leads to faster computation. To demonstrate the capabilities of the platform and experimentally prove its reliability and stability, 2 Satisfiability (SAT) problems were solved via 2 different methods. It is notable that this approach eliminated the need for PCR (Polymerase Chain Reaction) and enzymes, and as a result error rates and cost are decreased. Overall, this technique shows some significant advantages over previous experimental techniques such as short operating time, reusable surface and simple experimental steps. In Chapter 5, experimental demonstration of the feasibility of Hopfield Neural Network using DNA molecules can be seen. In this collaboration work with Professor Allen Mills and Dr. Dundar Karabay, we demonstrate the operation of six dimensional Hopfield associative memory storing various memories as an archetype fault tolerant neural network implemented using DNA molecular reactions. The results are read on an electronic microarray exploiting the semiconductor processing knowledge for fast and accurate hybridization rates.

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2. DNA as an Engineering Material in Assembly of Nanostructures

Abstract: DNA possesses inherent recognition and self-assembly capabilities. These unique features can be utilized for construction of functional materials as building blocks for nanoelectronics. We report the use of DNA towards the assembly and electronic functionality of nanostructures based on conjugates of carbon nanotubes (CNTs) and nanowires (NWs). To fabricate molecular-scale electronics, a novel approach to self-assemble arbitrarily complex nanostructures is proposed. The hybridization of complementary oligo sequences drives the formation of the designed nanostructures. Here Lock and Key LithographyTM is described as a method that provides strong information control to the system and provides the ability to form arbitrarily complex nanostructures. First, assembly of CNTs with DNA is demonstrated and electrical measurements of these nanoarchitectures show negative differential resistance due to CNT-DNA interfaces, which indicates a biomimetic route to fabricating resonant tunneling diodes. Second, DNA sequences to satisfy self-assembly are designed and tested on an electronic microarray platform for their selectivity. Gold NWs are synthesized through electrochemistry and addressed to specific locations within the microarray via functionalized oligos. At the last step of this project multi-component NWs are synthesized and functionalized with oligos, and end-to-end assembly of NWs is realized with designed DNA sequences. Our experimental results provide greater understanding of DNA as an engineering material and show the potentials of molecular electronics.

Introduction

The semiconductor industry is developing lithographic technology for feature sizes below 22 nm and exploring new classes of transistors that use CNTs or silicon NWs¹. A major goal of nanotechnology is to couple the self-assembly of molecular nanostructures with conventional microfabrication². This will enable us to register individual molecular nanostructures and integrate them into functional devices³. One strategy is to use the inherent recognition and self-assembly capabilities of DNA³. Many other research groups⁴⁻¹⁰ have studied whether biological materials can be integrated with nanoscale building blocks to construct higher-ordered structures. It has been demonstrated by Paul Rothemund that DNA can be folded into various shapes and forms¹¹. Due to their selectivity and recognition at the molecular scale, DNA molecules can offer advantages on nanometer-scale spatial control and self-assembly of multiple components onto a device. In this bottom-up approach molecules and their noncovalent forces are employed to exploit their selectivity in a way similar to parallel processing of many molecules. The objective is directed assembly of complex and functional nanomaterials with controlled size and composition^{6, 8-12}. DNA is an ideal molecule for constructing nanoscale structures because DNA can be programmed from the four nucleic acids: A (Adenine), T (Thymine), G (Guanine), and C (Cytosine). That property enables DNA research to be much more than genetic research¹³⁻¹⁷.

Among various nanostructures we chose CNTs and NWs due to their unique properties. One-dimensional nanostructures can be useful for future high-density nanoscale device

applications including sensors, optoelectronics and electronics¹⁸⁻²⁴. Functional hybrid materials aim to combine organic and inorganic components for their desirable properties and functionalities. The integration of NWs and CNTs with biological molecules can combine the electronic and mechanical properties of the NWs and CNTs with the self-assembly capability of biomolecules²⁵. The focus of the project presented here is DNA-based assembly of nanostructures with the goal of creating circuits with the building block of life. DNA is used towards the assembly of nanostructures. Electronic microarray platforms are employed for testing selectivity of DNA sequences, manipulating nanowires in the solution under electric field and making templates onto which discrete components can be addressed.

At first, we investigate the use of DNA towards the assembly and electronic functionality of CNTs. Second, DNA sequences to satisfy self-assembly are designed and tested on an electronic microarray platform for their selectivity. Gold (Au) NWs are synthesized through electrochemistry and DNA sequences are attached to the NWs, which are addressed to specific locations within the electronic microarray. Finally, end-to-end assembly of multi-component NWs is accomplished via designed DNA sequences.

In summary, DNA is used as an engineering material for self-assembly, as smart glue and as the medium for carrying NWs on to desired locations on the microarray due to DNA's negative charged backbone, inherent recognition and self-assembly capabilities.

Results and Discussion

Carbon Nanotubes

CNTs emerged as potential candidates for next-generation electronics due to their extraordinary strength and unique electrical properties as well as their thermal conduction efficiency²⁶⁻³¹. Various devices developed so far have major limitations in terms of precision, specificity and interconnection³¹⁻³⁵. This is one of the road blocks in their integration to large-scale devices and complex circuits. One proposed solution is self-assembly by molecular recognition. Nucleic acids have emerged as the most widely used molecules for self-assembly³⁶⁻⁴¹. This is due to the predictable self-assembly of complementary nucleic acid strands by Watson-Crick hybridization, the well-defined dimensions of nucleic acid duplexes and the opportunity of functionalization by conjugation⁴¹⁻⁴⁵. First, we use DNA towards the assembly and electronic functionality of single wall nanotubes (SWNTs).

SWNT-DNA-SWNT conjugates are synthesized using carbodiimide coupling chemistry^{46,47}. In this work DNA is used as a linker between SWNTs by functionalizing them at both ends. In this process, SWNTs from Sigma Aldrich are mildly oxidized using nitric acid. After oxidation the solution is filtrated and sonicated, and SWNTs are individualized via centrifugation⁴⁶. This process provides oxidized, clean, short and individualized SWNTs. Next, NHS esters are established at the oxidized sides of the SWNTs. DNA sequence is functionalized on both ends by amino acid residue. This sequence is mixed with SWNT solution and incubated. Carbodiimide coupling of SWNT-

NHS esters and DNA forms SWNT-DNA-SWNT conjugates^{46, 47} (Figure 2.1a). This is accomplished due to the amide bond formed between the amino-acid residue on DNA and SWNT bearing NHS esters. The resultant sample is pipetted and dropped on the Si substrate, which has pre-patterned metal electrodes (Figure 2.1b).

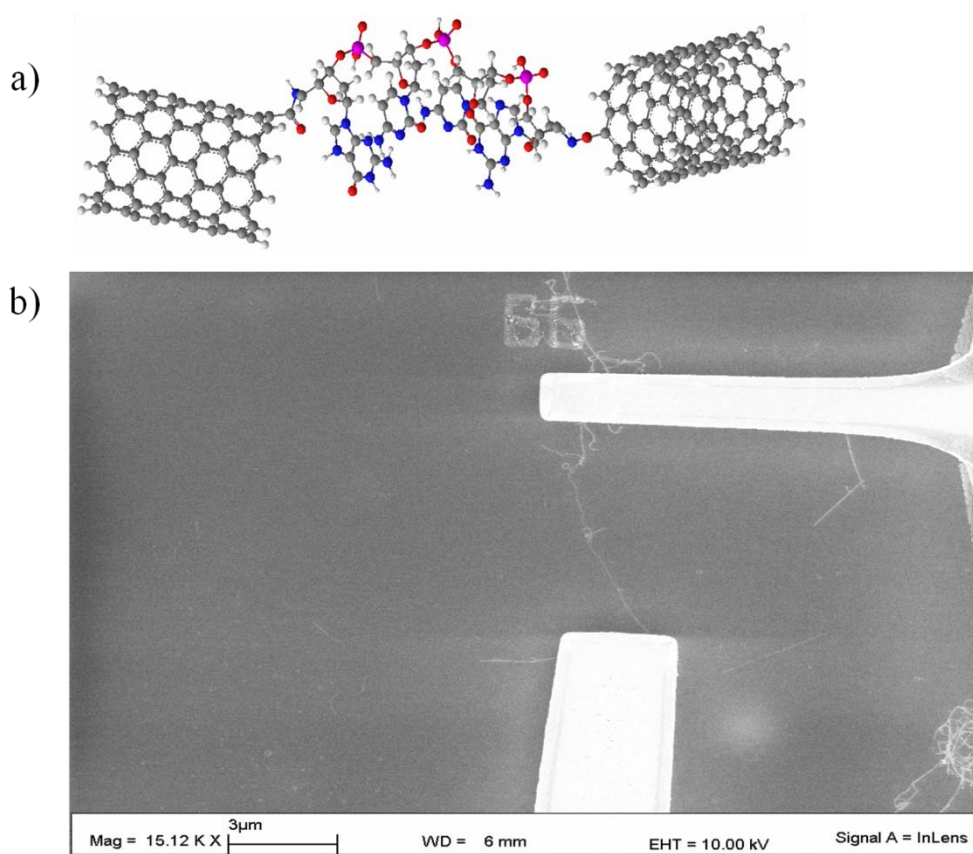


Figure 2.1: a) Illustration of SWNT-DNA-SWNT nanostructures where DNA connects two SWNTs b) SEM image of a SWNT-DNA-SWNT structure fixed between metal contacts.

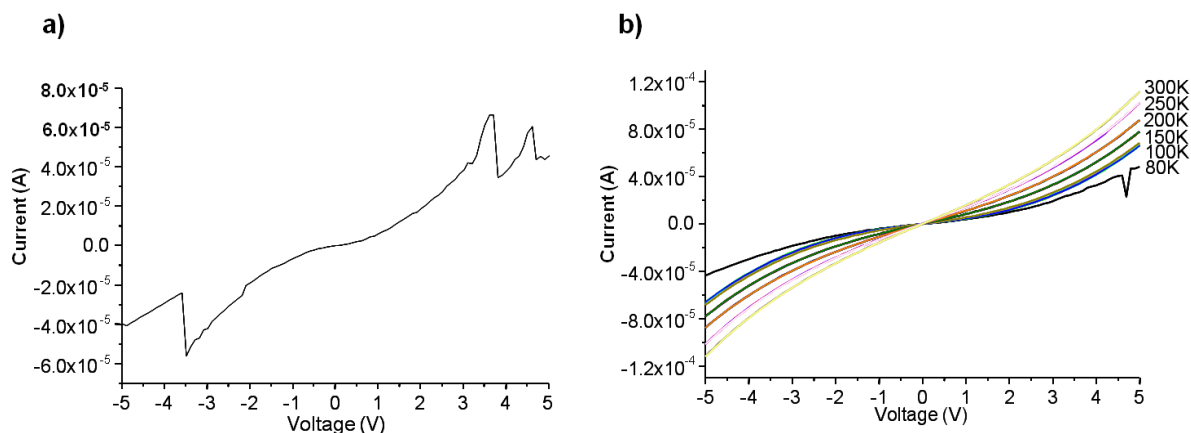


Figure 2.2: a) I-V measurement of SWNT-DNA-SWNT structure fixed between metal contacts b) I-V measurements of SWNT-DNA-SWNT from low temperature to room temperature.

Electrical measurements (I-V characterization) of the nanostructures demonstrate negative differential resistance (NDR) with the presence of SWNT-DNA interfaces (Figure 2.2a). This is a characteristic of resonant tunneling diodes (RTD). This can be interpreted as a biomimetic route to fabricating resonant tunneling diodes. Similar results were shown in other studies in our lab^{46, 47}. The addition here is a more robust signal and repeated results of the NDR effect due to mainly cleaner samples and better contacts. I-V behavior under different temperatures shows that conductivity of SWNT-DNA nanostructure varies with the measurement temperature. The conductivity increases when the temperature is raised (Figure 2.2b). The details of the experimental process are explained in the experiment section.

RTD response comes from the SWNT–DNA–SWNT entities because in the absence of DNA oxidized SWNTs do not show similar electrical data. Similar results are also acquired in our group where SWNT-DNA and SWNT-PNA nanostructures exhibited NDR effect⁴⁶⁻⁴⁸. Furthermore, Professor Roger Lake’s group demonstrated via

computations based on density functional theory (DFT) and non-equilibrium Green function (NEGF) approach the electron transport and RTD characteristics of these nanostructures⁴⁹⁻⁵³. These simulations reveal that the electron transport process along the SWNT-DNA-SWNT nanostructures occurs via hopping or band conduction⁴⁹⁻⁵³. SWNT-DNA interfaces reduce the electron transport along the conjugates due to low conductivity of DNA. The current levels in these experiments can be justified by a hopping process between localized states when an electric field is applied⁴⁷. Reasons for variation in conductivity and change of the nature of the I-V curve are: 1) variation in number of DNA molecules attached to the tip and or walls of the tubes will change the characteristics, and 2) bunching of metallic and semiconducting SWNTs in the solution, with no easy way to separate them.

We would like to mention that in other studies DNA sequences wrap around a CNT in solution due to the π stacking between the DNA bases and CNT^{6, 8, 54, 55}. In this case, the EDC coupling method avoids the probability of DNA units wrapping around the SWNT, because of the effective interaction of the amino group on DNA with the carboxylic group on the SWNT. Therefore, most of the DNA fragments are conjugated to the ends of the SWNTs via an amide group, which is desired for achieving proper and selective self-assembly of nanoarchitectures and devices.

DNA Sequence Design for Self-Assembly

In the second part of the project, DNA serves as a smart linkage to assemble NWs and address them to the desired locations. Here Lock and Key Lithography™ is described as

a method where the hybridization of complementary oligos drives the formation of the designed nanostructures. This idea provides strong information control to the system, and provides the ability to form arbitrarily complex nanostructures as proposed at Figure 2.3.

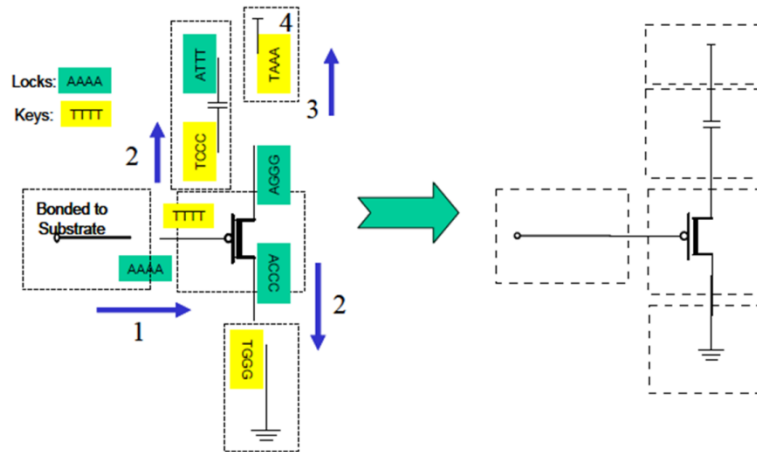


Figure 2.3: Lock and Key Lithography™ self-assembly components and procedures. The desired circuit structure is brought together via DNA hybridization.

The majority of self-assembly methods reported in the literature^{7, 36-41, 56,57} are suitable to form ordered, crystal-like structures. Current self-assembly paradigms generally lack the dense information content necessary to fabricate large, arbitrary structures. The design approach proposed here combines a unique molecular indexing scheme with molecular scale components to self-assemble structures of arbitrary complexity. This self-assembly system consists of three components: molecular-scale devices or interconnects; a number of DNA oligos named as “lock” sequences, and a number of complementary DNA oligos designated as “key” sequences. Specific DNA oligos are selected that effectively program the self-assembly process.

In this project we collaborated with Dr. John Hartley's group from SUNY Albany. The experimental work is carried out at UCR, and Dr. Hartley's group performed the simulation work. Computer programs were developed to simulate the propagation of the self-assembled network from the seed components to the entire nanostructure in Dr. Hartley's lab. Here these studies from Dr. Hartley's lab are summarized to give the reader a better picture of the project.

The process begins by placing seed components onto a substrate. The seed components transport and anchor several "lock" sequences on to the substrate. Details of these initial "locks" depend upon the design of the circuit. Next, the components that are adjacent to the seed components are introduced in solution. These components carry "key" sequences complementary to the "lock" sequences. The hybridization of "lock" and "key" sequences drives the components to the correct positions adjacent to the seed. Once these components are anchored the lock sequences are ready for the next step. The cycle repeats with new lock and key carrying components keyed to the locks on the substrate continuing the process (Figure 2.4).

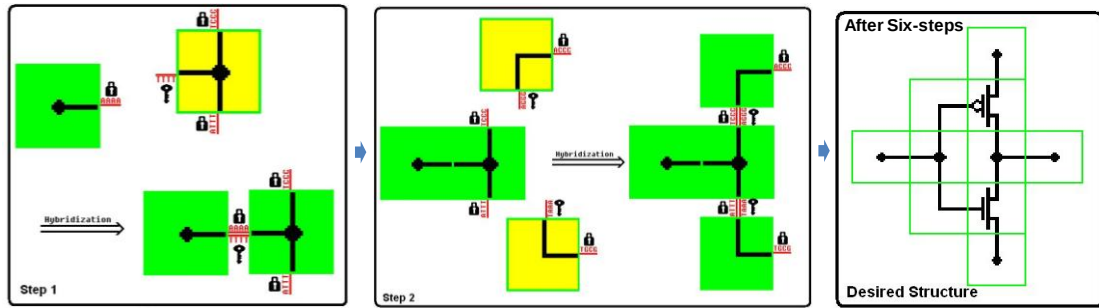


Figure 2.4: Self-assembly components and procedures. The desired structure in this example is an inverter circuit consisting of ten components. Green blocks in this figure represent components that are already anchored to substrate, while yellow blocks represent components to be introduced in the current step, and they are mobile in a solution. This inverter circuit can be built using Lock and Key LithographyTM in six steps.

The Feasibility of Lock and Key LithographyTM

Large numbers of pseudo-random structures are examined, and both the number of steps of self-assembly and required unique lock and key sequences are calculated. Results are reported for the average of these structures. The simulations also examined how the position of the seed component affects the number of process steps and the number of lock and key pairs required. It was observed that a smaller number of steps was required when starting near the middle of a structure, while fewer unique lock and key sequences are needed when starting from the corner or edge. As might be expected, both the number of steps and lock/key pairs varied as the square root of the number of components with some dependency on the location of the starting seed. This suggests that the position of seed component can be optimized either in favor of fewer steps or as a reduction of the number of lock and key sequences required (Figure 2.5).

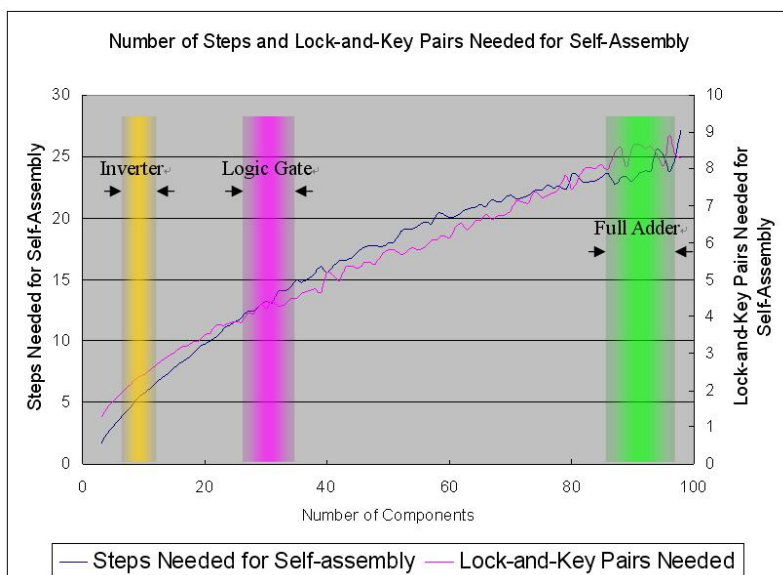


Figure 2.5: Steps of self-assembly and number of lock and key sequences versus the complexities of the electronic circuit. A single component in this apparatus could be implemented using a nanowire, nanotube or other molecular device, and it usually corresponds to a transistor, a wired connection, or an input/output contact. An inverter circuit typically requires 10 components (as shown in Figure 2.4), while a logic gate requires approximately 25 to 30 components, depending on the function of the logic gate. A full adder can be implemented using 90 to 100 components. All three cases are marked with the colored regions in the figure. An early age 8-bit microprocessor has 2300 to 2500 transistors, and would require approximately 10,000 to 12,500 keyed components in a self-assembly process such as described here.

Controlling Error Rates in Lock and Key Lithography™

Errors occur when a lock is jammed with the wrong key. It is possible that a key becomes partially hybridized with a mismatched lock preventing the correct hybridization. Since the hybridization/bonding is weaker in pairs formed by mismatched sequences relative to pairs formed by fully complementary sequences, it is possible to design an error correction scheme utilizing this difference in hybridization strength.

Hybridization strength can be characterized here with melting temperature (T_M) of the sequences. The T_M of a perfectly matched oligo pair is higher than a mismatched set.

Lock and key sequences are chosen such that the T_{MS} (melting temperatures) of perfectly matched oligos are higher than imperfectly matched pairs. T_{MS} of the DNA pairs are calculated using nearest neighbor thermodynamics⁵⁸. During the selection of the DNA sequences, T_{MS} for perfectly matched pairs are designed to be higher than a threshold temperature, namely, T_H (higher T_M threshold). T_{MS} for all possible mismatched pairs are chosen to be lower than another threshold, namely, T_L (lower T_M threshold).

In addition to the above requirements, the designed DNA sequences should not form stable secondary structures above T_L . Otherwise lock sequences may form stable structures that can block the key sequences and vice versa. Also the T_M of helices formed by two identical key sequences should be below T_L . This requirement prevents key sequences to be self-complementary. This prevents the stable helices formed by identical key sequences.

In this study sequences of 8 base pairs (bp) in length are examined for self-assembly. Out of the large number of available DNA sequences, (65,536 ($=4^8$) DNA sequences of 8bp in length), a limited number of lock and key sequences satisfy the above requirements. A set of 24 pairs of lock and key sequences that satisfy the above requirements, with $T_H=45C$, and $T_L=10C$, are designed. The larger the difference between T_H and T_L , the easier it is to eliminate errors through temperature controlled washing steps with the electronic microarray platform. The 24 perfect matches formed by complementary oligo sequences melt above $T_H=45C$. Meanwhile, the 2280 ($2280 = (2 \times 24)^2 - 24$) mismatched pairs are predicted to have melting temperatures below $T_L=10C$.

With the selection of sequences fulfilling these requirements, it is possible to apply temperature controlled wash cycles as the stringency conditions on the microarray and “erase” errors. Because all of the mismatched lock and key pairs can be eliminated with temperature controlled washing cycles, the perfect matching sequences may be obtained at the end of the experiments. When the temperature of the self-assembly environment is adjusted to a temperature between T_L and T_H , the mismatched keys release from the locks and can be rinsed away while all of the perfectly matched pairs remain hybridized. The experimental verifications of these theoretical predictions are revealed in the coming pages of this document. Figure 2.6 shows the predicted melting temperatures of the 24 DNA pairs. It is shown that the perfectly matched “lock” and “key” sequences have T_M s above 45C while the highest T_M of possible mismatched pairs are below 10C. With these 24 pairs of DNA sequences, we try to address circuit components or interconnects to build up complex nanostructures.

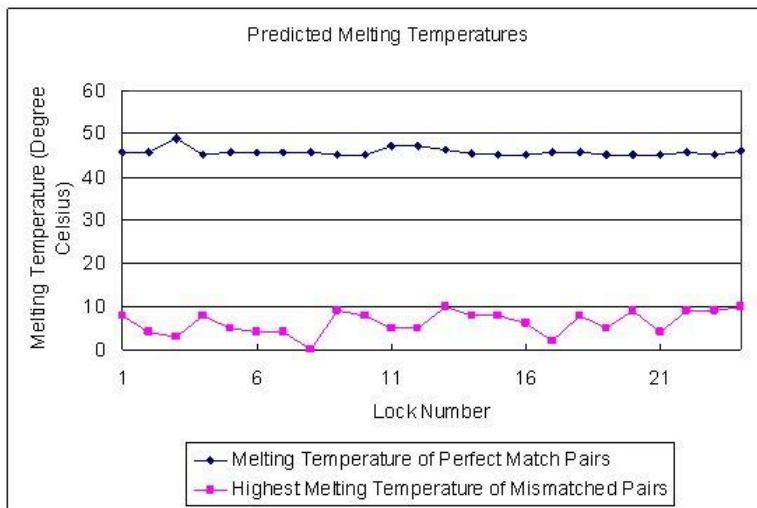


Figure 2.6: Predicted melting temperatures of DNA helixes.

Experimental Results of Selectivity of the Designed DNA Sequences

The next step in the project is to verify the simulation results. The selectivity of the sequences is tested on an electronic microarray to see how well the oligos can satisfy the assembly. An electronic microarray platform with 400 individually addressable sites is utilized for this purpose. The details of the experiments as well as the operation of the microarray are explained in the experimental section. Basically, the working mechanism of the microarray is based on complementary hybridization of matching DNA strands. 200 nano Molar biotinylated lock sequences are attracted one at a time to the streptavidin containing pads via application of 600 nano Ampere constant current at a particular pad. After immobilization of a sequence, the array is washed with a histidine buffer. With this method 24 lock oligos are immobilized on the microarray and one key oligos' selectivity against 24 lock oligos are investigated. This experiment is carried out for all key sequences to determine their selectivity. Each row in Figure 2.7 represents 24 lock sequence immobilized on the microarray and one key sequence is introduced to the array to determine the selectivity of that sequence. Figure 2.7 shows the image taken with a CCD camera as well as the fluorescent signal strength measurements from each pad proving the selectivity of the sequences. To obtain the results presented below, key sequences are introduced to the array with reporter sequences hybridized at the end of the keys. Reporter sequences contain a green fluorophore and fluorescence from a particular pad means hybridization. The fluorescent signal proportional to the concentration of lock-key- strands is measured. The microarray is heated to an oligo specific temperature and washed with a high salt buffer (50mM sodium phosphate and 500mM sodium chloride

pH7.0) before imaging the array with a CCD camera. The mis-hybridized oligos as well as unbound DNAs are washed away, and the bound DNA complexes remain. The fluorescent signals are measured from each pad to verify correct hybridization. After completion of the hybridization of “lock” and “key” sequences, fluorescent imaging is used to quantify the selectivity. The selectivity of the designed oligos can be seen from the high fluorescent signal strength coming from correctly-hybridized pads and the easily eliminated mis-matched sequences.

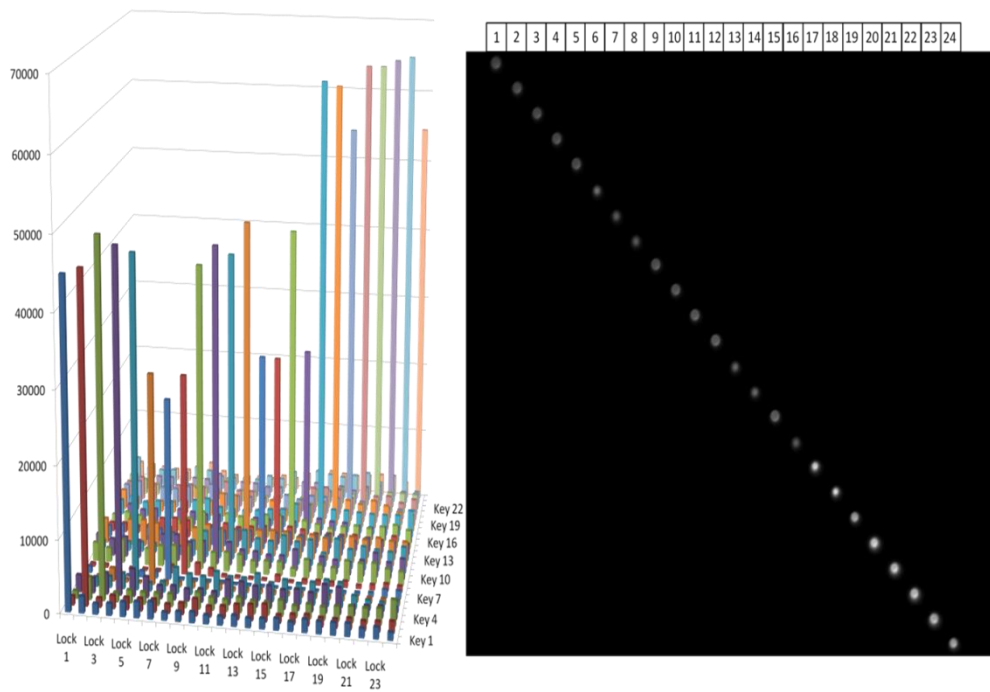


Figure 2.7: Experimental results for 24 lock and key sequences. Signal strength in bar graphical form on the left is in relative fluorescent unit (rfu). Grayscale form recorded via a CCD camera is seen on the right. Each row in both figures represents 24 Lock sequences immobilized on the microarray. Afterwards one key sequence is introduced to the array to determine the selectivity of that sequence. Same data in both forms confirm the selective hybridization of the designed lock and key sequences. The high selectivity of the sequences can be seen from the high fluorescent signal coming from the correctly-hybridized sequences and the easily eliminated mis-matched sequences.

In our experiments, “selectivity” is defined as the matching lock and key sequence signal divided by the highest non-specific signal coming from other pads when introduced one key at a time. That parameter shows us in the worst case scenario how much non-specific matching can be eliminated and how much remains after all the temperature controlled washing cycles.

The conclusion after several selectivity experiments is as follows: The average mismatching hybridization signal value is on the order of 2100 rfu (ranging between 1000 to 3000 rfu), the matching lock-key sequence signals are 10 to 22 times more than the maximum mis-hybridized lock-key sequence (mostly around 20). Temperature controlled washing of the array in order to reduce binding errors is repeated several times according to the key sequence introduced to the immobilized locks. During investigation of the selectivity of a particular key, wash temperatures were between T_L and T_H . With this approach, the mismatched keys are eliminated and perfectly matched lock and key sequences remain attached. This process demonstrates that we can have a very high ratio of matching lock-key pairs to mismatched pairs. In a biological experiment in this nature, the expected ratio of correctly hybridized sequence to mis-hybridized sequence is around 5. After a number of washing cycles between T_L and T_H of the sequences, a clear signal from the matched lock and key sequence was obtained for each sequence. This demonstrates integrated error correction function to remove the excess non-selectively bound (mismatched) keys.

Electrochemically Synthesized Gold Nanowires Addressed on to the Microarray via Functionalized DNA

After demonstrating the selectivity of the 24 lock-key sequences, the next step is synthesizing the nanostructures. To study the feasibility of the idea, gold (Au) NWs were fabricated electrochemically using anodic alumina templates (see experimental section for process details and Figure 2.7a for SEM image of the Au NWs). These NWs were selectively addressed to specific locations within the electronic microarray via DNA. Thiol molecules establish a monolayer on the Au surface⁶⁰⁻⁶². We functionalized various key sequences with thiol at their 5' ends. Thiolated key sequences are attached on Au NWs, which are around 1µm in length and 200 nm in diameter (Figure 2.7b). In this study the key sequences were found to cover the entire length of the NWs as expected. We functionalized Au NWs in 3 different vials with 3 different key sequences, namely key-1, key-2 and key-3 (Figure 2.7b). In addition to thiol, each key sequence has a TET molecule at its 3' end for fluorescent detection. The functionalized Au nanowires were driven to the desired pads on the array by application of 60 seconds of current at 800 nano Amperes. The result of the experiment is seen in Figure 2.7c. Selective addressing of NWs with specific lock-key combinations is demonstrated for three different sets. In Figure 2.7d the step by step process of addressing functionalized Au NWs can be seen. Lock-1, lock-2 and lock-3 are immobilized on to each row while row#4 in each 4 by 4 fluorescent image is left empty (without any oligo) for control purposes. Key sequence functionalized Au NWs are manipulated inside the solution due to the charged backbone of DNA and addressed on to the desired location with the electric field. Afterwards, when

the array is washed, signal comes from correctly hybridized pads, and the mis-matched hybridizations are eliminated. This demonstrates that functionalized NWs are addressed on to the pads with the applied electric current due to the charged backbone of the oligos. The dielectrophoretic (DEP) manipulation of the DNA functionalized NWs are not affected by the precipitation or diffusion in the solution.

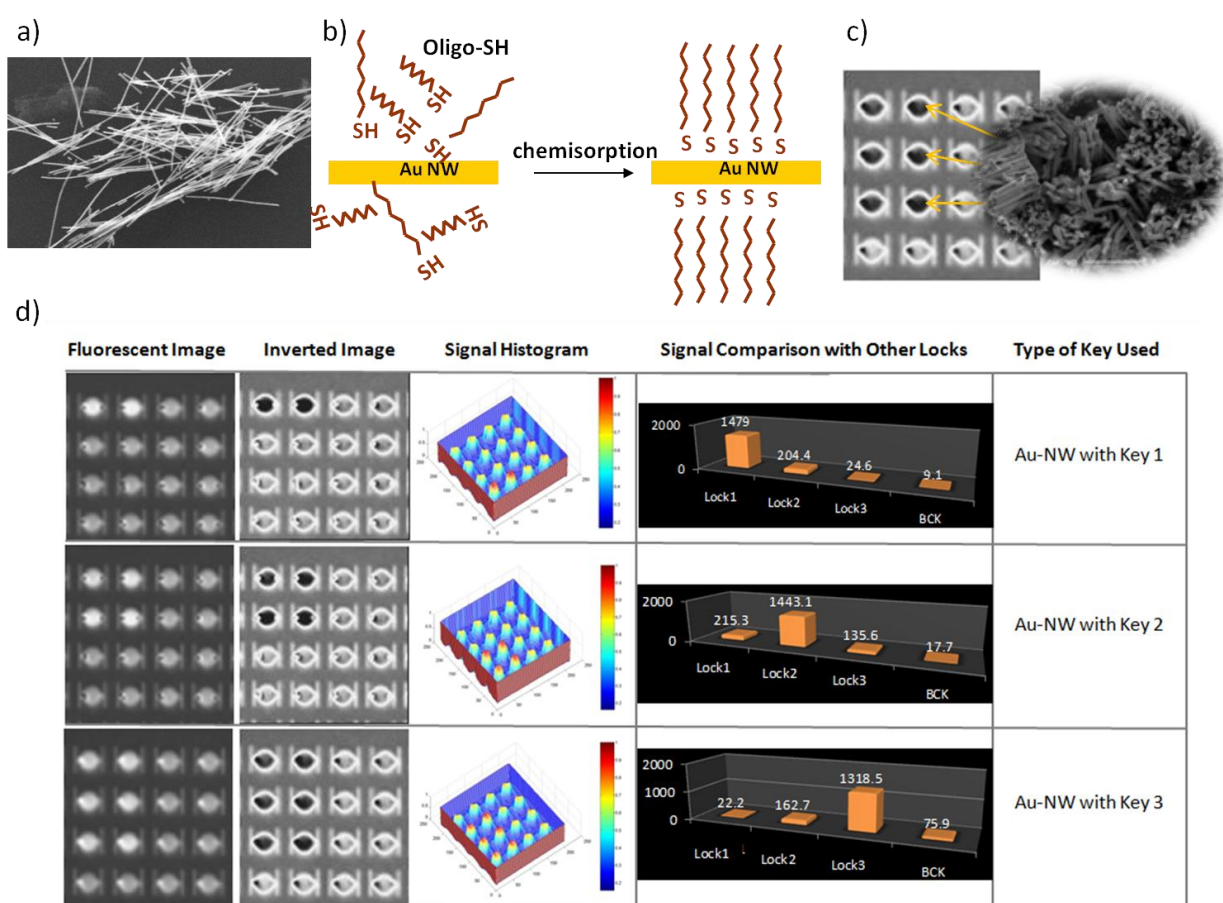


Figure 2.8: a) SEM image of the electrochemically synthesized Au nanowires b) Oligo functionalization of Au nanowires c) Dielectrophoretic (DEP) manipulation of NW-DNA structures and addressing them on to the microarray via electric field. d) Experimental results for step by step addressing of Au NWs onto the microarray utilizing the lock and key™ methodology. Here the NWs in each row carry a different key sequence and a fluorophore to enable optical detection. Gray scale fluorescent image, inverted image for clarity, 3D-histogram demonstrating the signal strength compared with the neighboring pads and comparison of signal strength between the complementary and non-

complementary lock-key nanowire combinations are demonstrated for three different keys functionalized Au nanowires. Only specific key-functionalized Au nanowires are addressed to the specific/desired lock containing pads on the microarray.

End-to-end Assembly of Multi-component Nanowires via DNA

At the final stage of this project, to enable end-to-end assembly of nanowires, Au/Ni/Ag multisegment NWs are synthesized electrochemically. The fabrication of the NWs is explained in the experimental section. After the synthesis of multi-component NWs, thiolated key and lock sequences are mixed in different vials with the suspension of NW solutions (Figure 2.9). To achieve functionalization and hybridization, lock and key sequences modified with thiol groups and TET molecules (for fluorescent detection) were diluted to 600 $\mu\text{g/mL}$ in TE buffer (pH=8). The adsorption of thiols on different metal surfaces, in this case Au and Ag, has been well studied, and it has been proved that thiol groups bind to Au and Ag surfaces strongly⁶⁰⁻⁶². Meanwhile, a nickel (Ni) segment is employed as thiol-resistant segment^{63, 64}. Multi-component NWs are selectively functionalized by the lock and key oligos through specific binding of thiol group to the gold and silver segments. During this process, 100 μl lock and key solutions were added into 100 μl suspensions of Au/Ni/Ag NWs separately (Figure 2.9). The resulting mixtures were vortexed for 24 hours and then were centrifuged at 13000 rpm for 120 min. Supernatant was removed to avoid excessive oligos. Precipitations were suspended again in TE buffer and two solutions were mixed together. When a suspension of nanowires functionalized with thiolated lock sequences was mixed with an equal volume of nanowires which were functionalized by thiolated key oligos, end-to-end assembly is

achieved (Figure 2.10). Lock and key sequence functionalized NWs are hybridized and this process provides the NW-DNA-NW nanoarchitectures (Figure 2.10).

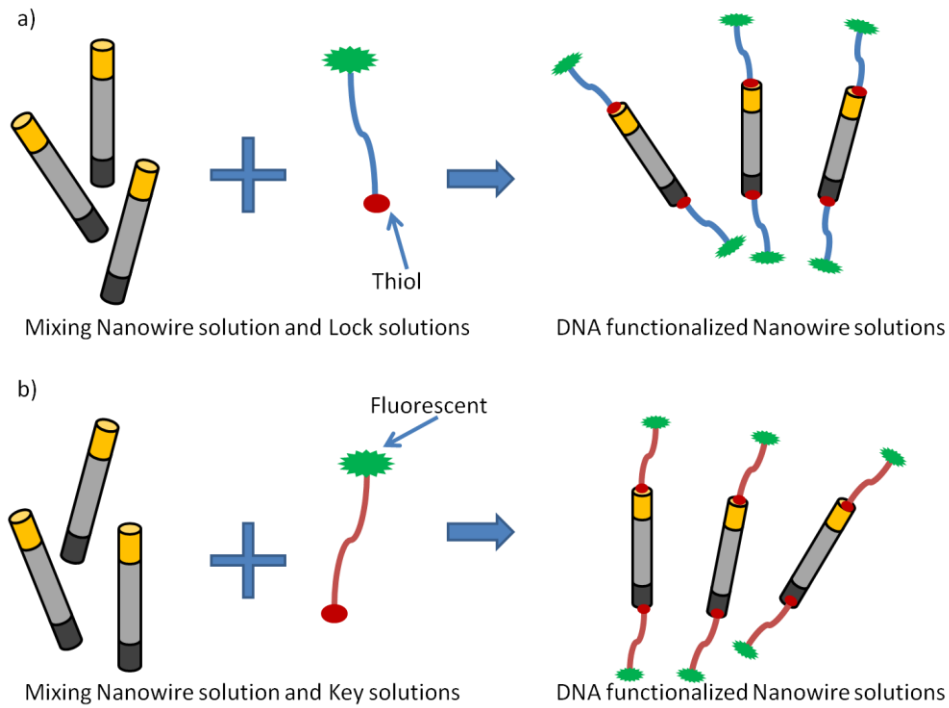


Figure 2.9: Au/Ni/Ag multisegment NW functionalization with DNA sequences. a) Multisegment (Ag/Ni/Au) Nanowire solution is mixed with thiolated lock sequences. After incubation thiolated sequences attach to the Au and Ag segments of the nanowires. b) Here Multisegment (Ag/Ni/Au) Nanowire solution is mixed with thiolated key sequences.

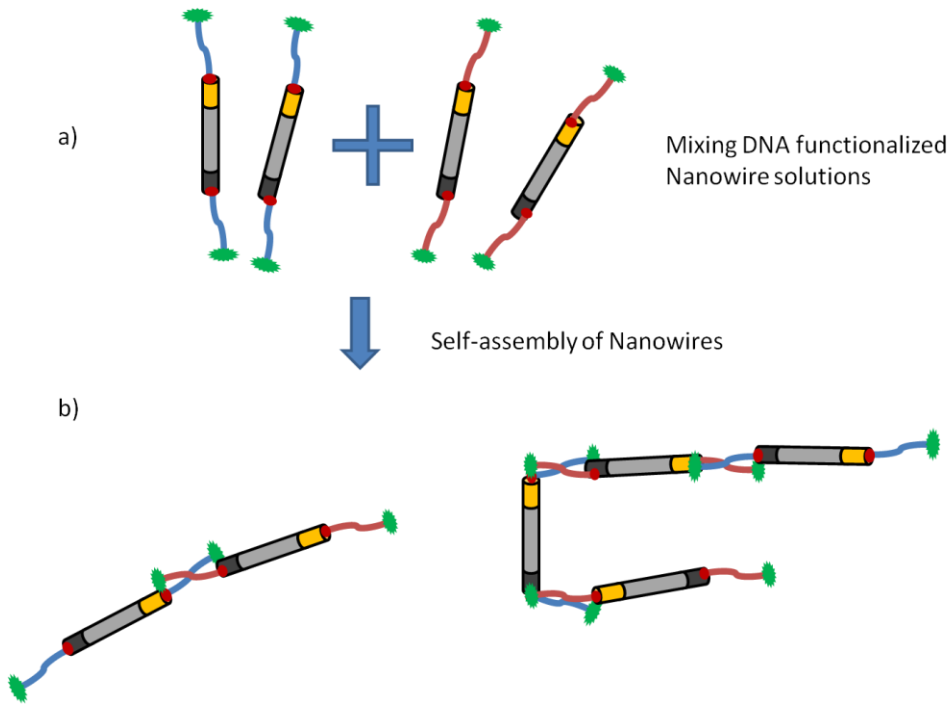


Figure 2.10: Self-assembly of DNA functionalized Au/Ni/Ag multisegment NWs. a) Lock and key functionalized Nanowires are mixed. b) Self-assembly via DNA resulted to the NW-DNA-NW structures and the chains of these structures.

The result of the self-assembly is confirmed with the SEM images (Figure 2.11a to c).

The NWs that have Au and Ni segments only (some NWs during fabrication lose their Ag parts or some pores don't have Ag diffused deep enough or broken therefore have only Au and Ni) show NW-DNA-NW configurations (Figure 2.12) while NWs that have Au/Ni/Ag segments show the NW-DNA-NW-DNA-NW type chains (Figure 2.11a to c). Finally, DNA-directed self-assembly of Au/Ni/Ag NWs at various lengths is accomplished. The NWs fabricated here is not device quality, but we have proved that Lock and Key Lithography can be used to bring/assemble the NWs together with DNA.

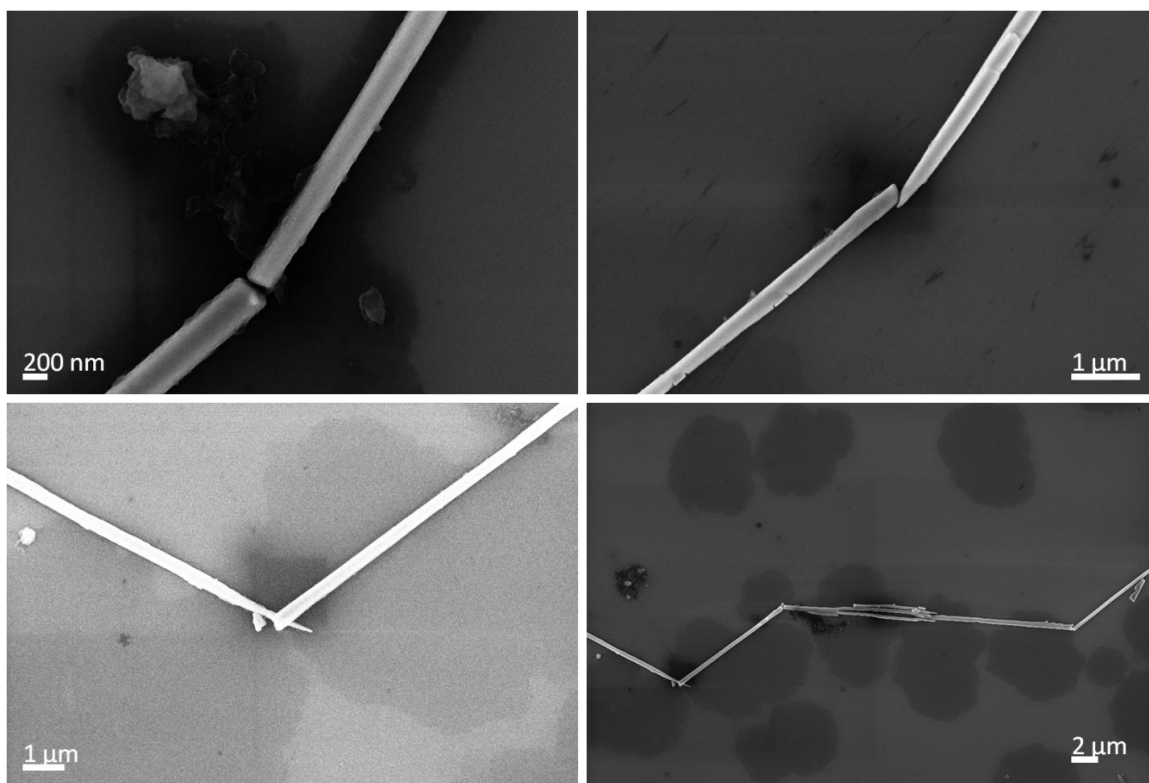


Figure 2.11a: DNA-directed self assembly of Au/Ni/Ag nanowires SEM images.

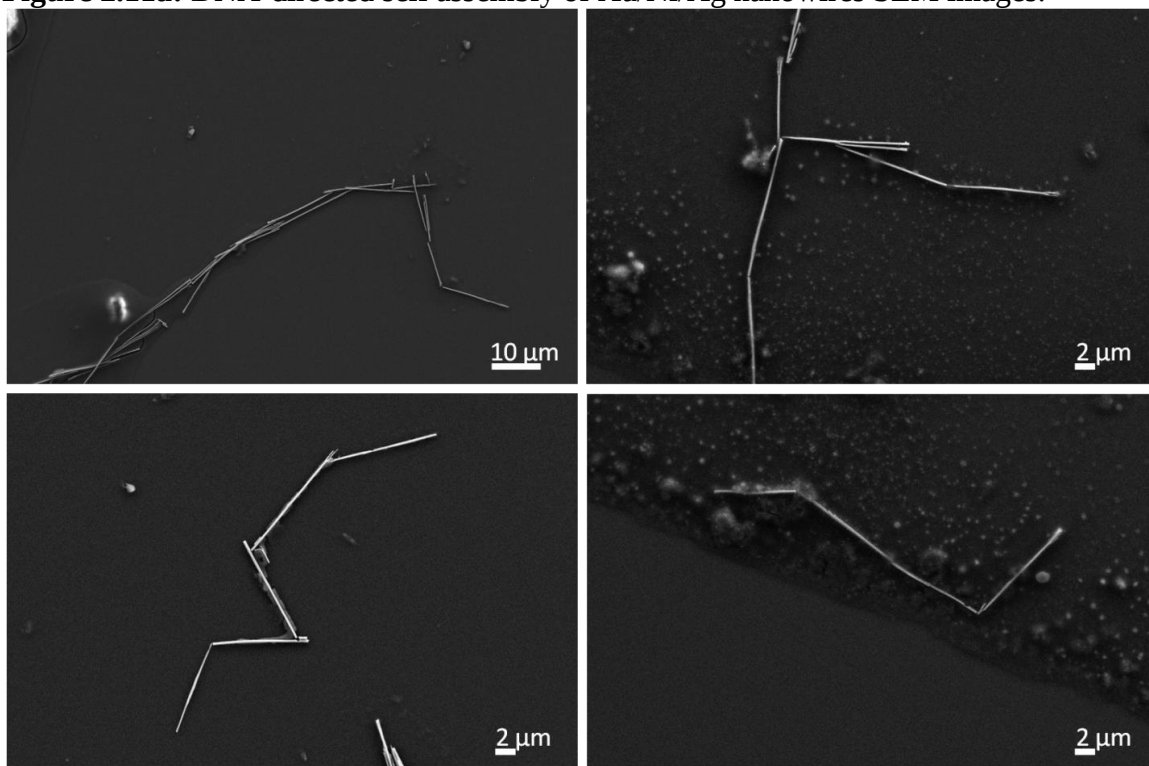


Figure 2.11b: DNA-directed self assembly of Au/Ni/Ag nanowires SEM images.

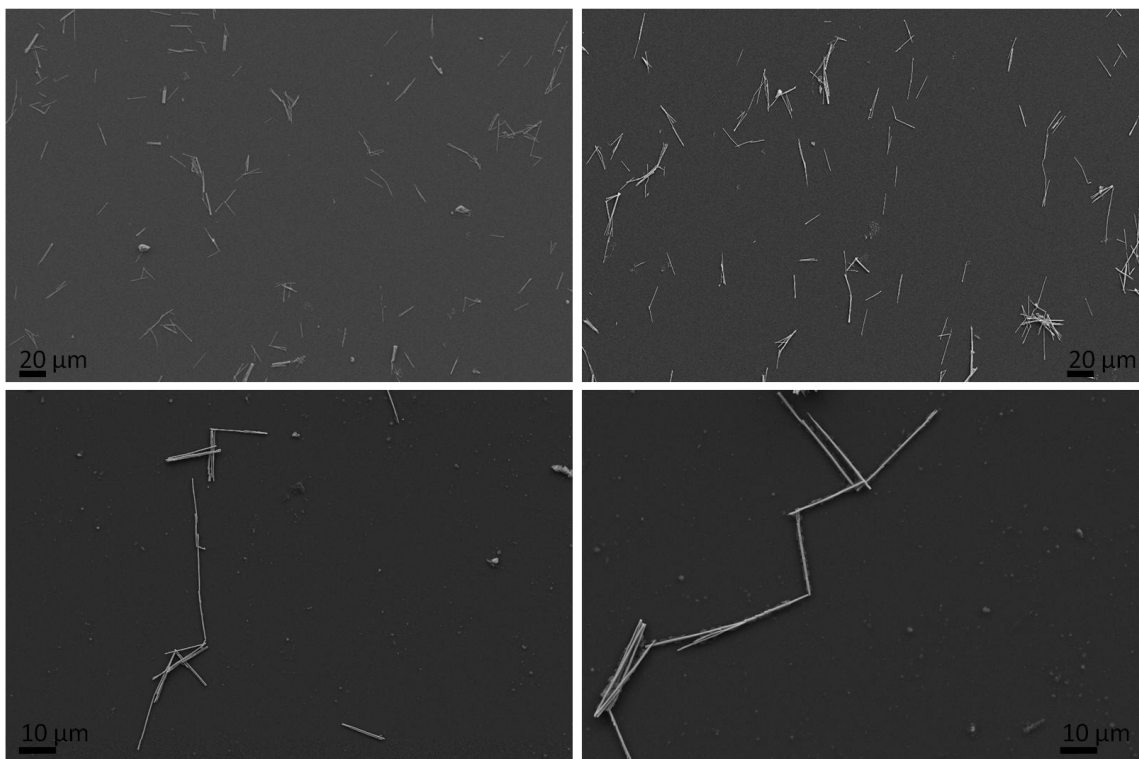


Figure 2.11c: DNA-directed self assembly of Au/Ni/Ag nanowires SEM images.

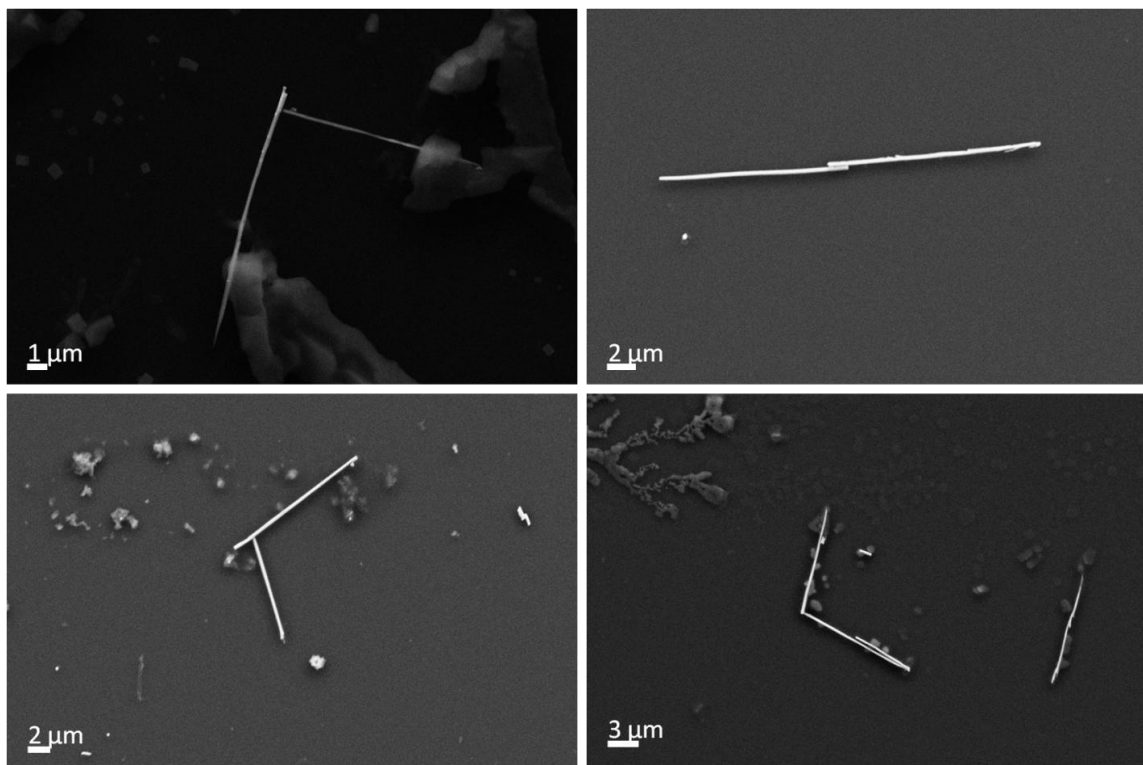


Figure 2.12: DNA-directed self assembly of Au/Ni nanowires SEM images.

DNA's molecular recognition and self-assembly properties as well as its negative charged backbone are utilized to prove the feasibility studies proposed by the Lock and Key LithographyTM.

Conclusion

Molecular electronics can be defined as technology where electronic functions are performed by single molecules, small groups of molecules, CNTs, metallic or semiconducting NWs^{47, 65-67}. Manipulating individual molecules or particular groups of molecules to do useful tasks and using the electronic properties of these molecules as alternative next-generation device concepts is the primary aim of molecular electronics. This aim is important because of the limitations we face in the current technology with conventional silicon-based devices¹. We used DNA in our research for non-biological purposes and utilized the inherent recognition and self-assembly capabilities of DNA for nanoelectronics^{47, 48}. CNT and NW based bioconjugates are suitable candidates for molecular electronics because they incorporate the excellent electrical and structural properties of the nanostructures and the self-assembly properties of biomolecules⁴⁷. DNA's inherent recognition and self-assembly capabilities are utilized here to functionalize SWNTs. In this study the assembly and electronics functionality of SWNT-DNA conjugates are investigated. SWNTs functionalized with carboxylic groups at both ends are introduced to DNA sequences possessing amino terminal groups and hybridized through amide linkages. We demonstrate the successful synthesis of SWNT-DNA-SWNT and started to investigate their electrical characteristics for molecular electronics

applications. Dielectrophoretic (DEP) manipulation of NW-DNA structures and their addressing on to a microarray as well as end-to-end assembly of multi-component NWs are realized. When an electric field is applied on the microarray, DNA functionalized NWs within the solution are carried to the desired pads on the microarray and the hybridization of complementary oligo sequences drives the formation of the designed nanostructures. This is due to DNA's negative charge. We showed here the feasibility study of Lock and Key LithographyTM concept for future nanoelectronics applications. Our studies illustrate that the area of bio inspired assembly presents many new avenues toward the exciting future of nanoelectronics and nano biotechnology.

Experimental Details

This section provides a detailed description of the experimental procedures implemented in this project with commentary.

SWNT-DNA-SWNT Conjugate Synthesis

CNTs have been used to realize many molecular-scale electronic devices in transistor arrays, logic circuits or sensors, especially to utilize their size and superior electrical properties⁶⁸⁻⁷⁰. I had the opportunity to learn in our lab to grow CNTs in CVD and dispersed them with many different methods (Figure 2.13)^{47, 48}. Different biological molecules having molecular recognition properties are searched, but nucleic acids are one of the most widely used molecules for self-assembly⁷¹⁻⁷⁵. This is due to the predictable self-assembly of complementary nucleic acid strands by Watson-Crick hybridization, the

well-defined dimensions of nucleic acid duplexes and the opportunity for functionalization by conjugation.

Here we report the synthesis of SWNT-DNA-SWNT conjugates using carbodiimide coupling chemistry. In this work DNA is utilized as a linker between SWNTs by functionalizing them at both ends. The goal here is to develop functionalized SWNT-DNA-SWNT conjugates with molecular recognition which can be used to develop higher order assembly for molecular level electronics in the future.

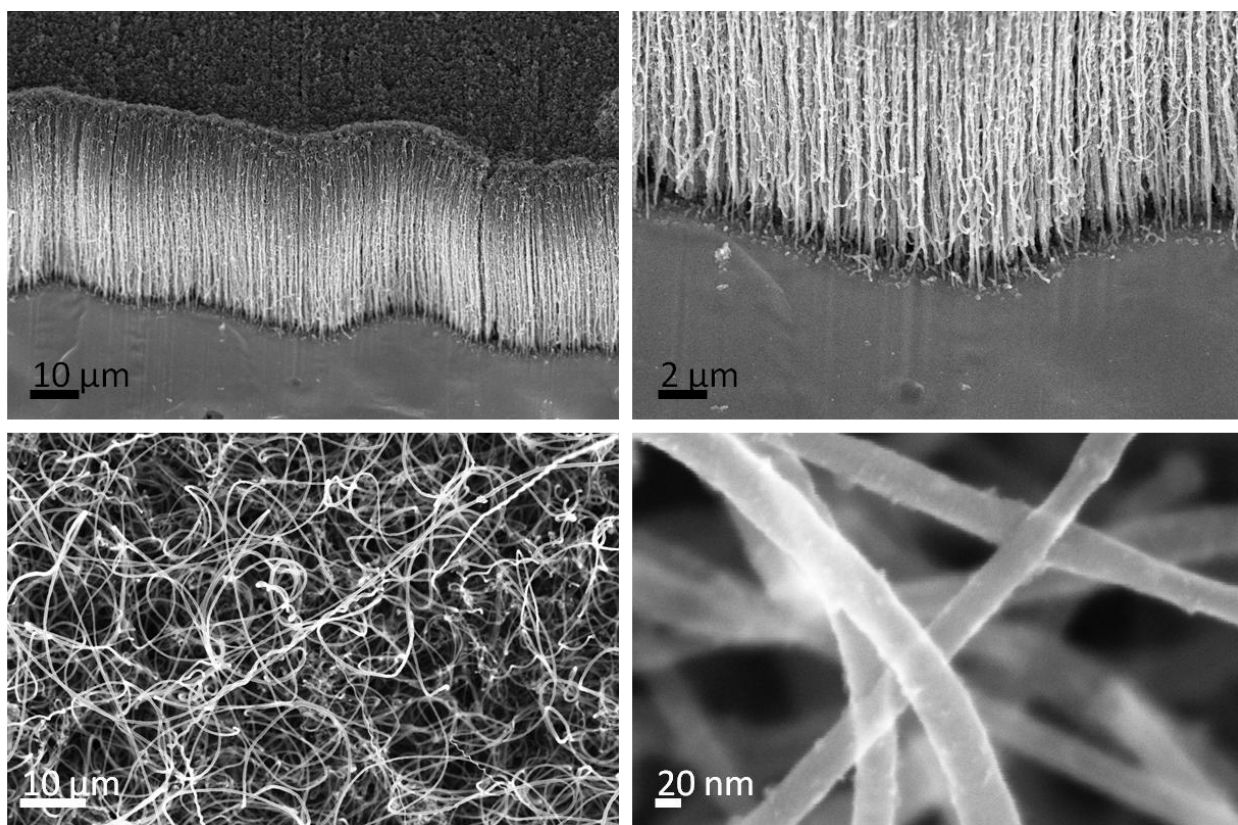


Figure 2.13: Carbon nanotubes produced in the lab. From growth to dispersion of the tubes many methods have been used.

The steps of experiment are as follows:

Oxidation of SWNTs

At first, SWNTs are mildly oxidized using nitric acid (HNO_3). Mild oxidation damages the side walls of the SWNTs as little as possible. Damages on the side walls of the SWNTs adversely affect the electron transport on the SWNTs. The oxidation conditions vary according to the producer of the SWNTs due to the reduction of impurities in SWNT structure. In this process, we used SWNTs from Sigma Aldrich instead of the SWNTs grown in the lab for better control of impurities. SWNTs are mildly oxidized using nitric acid (1M HNO_3 for 12 hours at 135C)^{9, 46, 76}. The nitric acid solution and SWNTs are introduced to a round bottom flask, and this flask is put in a hot oil (silicone oil) bath to maintain the temperature. The flask in the oil bath is put on top of a hot plate at 200C, and the oil bath temperature of 135C is obtained. A rotating stirrer at 200 rpm is used to make sure oil bath temperature is uniform. The oxidized SWNTs are seen as soluble as reported in other studies⁷⁷⁻⁷⁹.

Filtration of SWNTs

After oxidation, the solution cooled down to room temperature, filtrated with 200nm pore size PC membranes two times and sonicated⁴⁷. Filtration to remove the acid is done with DI water 10 times. Afterwards, SWNTs from the PC membrane are collected.

Shortening and Individualization of SWNTS

For further shortening, SWNTS are sonicated at 45C-55C at 40KHz for 2.5 hours in a 3:1 mixture of concentrated H_2SO_4 (98%) and HNO_3 (70%)^{46, 47, 77} as suggested in the literature. After this sonication SWNTs are mixed with water for dilution so they can be filtered easily with a PC membrane. After the second filtration SWNTs are individualized

via centrifugation at 13600 rpm for 180 minutes. This process reveals oxidized, clean, short and individualized SWNTs^{46, 47}.

Preparation of SWNT-NHS Esters

The next step is the establishment of NHS esters at the oxidized sides of the SWNTs. To establish that, SWNTs are incubated in 2mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, M.W. 191.6) and 5mM Sulfo-N-hydroxysuccinimide (sulfo-NHS, M.W. 217.3) for 30 minutes. As reported before⁸⁰, these esters are formed by conventional nucleophilic addition mechanism.

Preparation of DNA Solution for Conjugation

DNA sequence (NH₂- CGTCGCGC -NH₂) is functionalized on both ends by amino acid residue.

Carbodiimide Coupling of SWNT-NHS Esters and DNA to Form SWNT-DNA-SWNT Conjugates

The above sequence is brought to a final concentration of 100 micro molar, mixed with SWNT solution and incubated 5 hours. Carbodiimide coupling of SWNT-NHS esters and DNA form SWNT-DNA-SWNT conjugates (Figure 2.14). This is accomplished due to the amide bond formed between the amino-acid residue on DNA and SWNT bearing NHS esters. The resultant sample is pipetted, dropped on the Si substrate and connected with metal electrodes (Figure 2.1b).

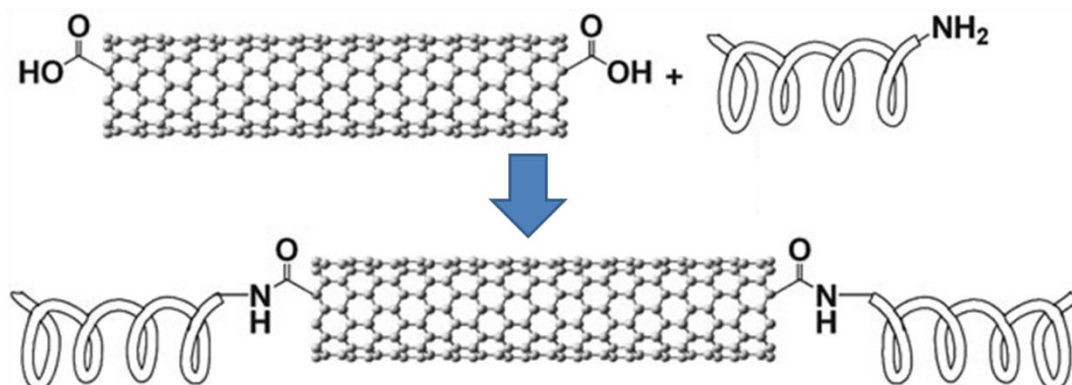


Figure 2.14: Conjugation of carboxylic groups of the SWNTs and amino groups of the DNA sequences.

The major problems faced were aggregation and high amount of impurities in the samples. Filtration is performed by centrifugation several times during the experimental steps. At the end of the SWNTs many carboxylic groups are generated following the oxidation process; this leads to a large number of DNA strands conjugated by amide linkage. In addition, DNA exists in solution in a coiled form, and aggregation and entanglement of DNA molecules are seen.

Final solution containing SWNT-DNA-SWNT conjugates were prepared by pipetting the mixture in to a new tube. Due to mild oxidation utilized in the process predominant end functionalization of SWNTs is observed^{46, 47}. Therefore, the attachment of the DNA is mostly at the ends of the SWNTs. SEM images of SWNT-DNA conjugates are collected at 5kV accelerating voltage in order to avoid damaging DNA by electron beam. The I–V characteristics are measured at ambient temperature in a dark environment using a Keithley 4200 semiconductor parametric analyzer system.

Experimental Setup for Testing Designed DNA Sequences

Figure 2.15 a-d illustrates the experimental setup for testing the designed DNA sequences selectively hybridizing “key” sequences to their complementary “lock” sequences. The figure shows 2 lock sequences. An electronic microarray platform with 400 individually addressable sites is utilized for this purpose as shown in Figure 2.15 e-f in detail⁸¹.

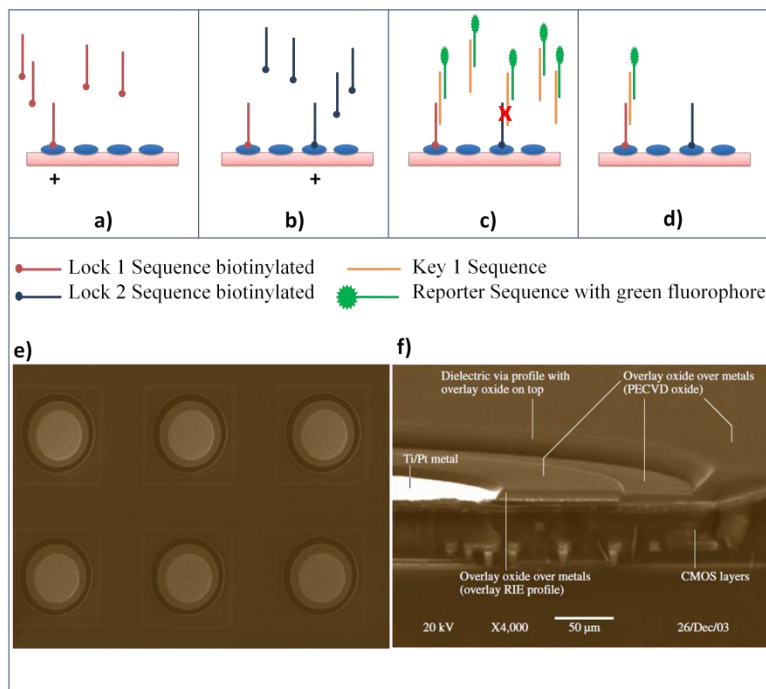


Figure 2.15: a to d: The experimental setup and working mechanism of the microarray, which is using complementary hybridization of matching DNA strands. Lock sequences are attached to the pads one at a time. Key sequence with the fluorophore reporter hybridized to it for optical recording is introduced to the array. Mismatched hybridizations marked with X are eliminated after temperature-controlled washing of the array and correctly hybridized complementary sequences remain. e) The CMOS chip has an array of 16×25 (400) sites (pads)⁸¹; each electrode is 80 μm in diameter with a 150 μm center-to-center distance. f) Cross-section of an individual microelectrode and the underlying CMOS circuitry⁸¹.

In Figure 2.15a, the microarray is exposed to a solution containing biotinylated lock 1 oligos. The pad is positively charged, attracting oligos and binding them to the pad.

When the charge is removed and the microarray is flushed with histidine buffer (100 mM histidine, 150 mM 1-Thioglycerol) and water solutions (0.05% ProClin 300 in water), the bound sequences remain. In Figure 2.15b, the microarray is exposed to a solution containing biotinylated lock 2 oligos and applying charge on a different pad immobilized lock 2 oligos to that pad. The unattached and excess oligos are removed when the array is washed with histidine buffer⁸²⁻⁸⁴. To immobilize each DNA strand on the platform, 600 nano Ampere constant current on each pad is applied for 40 seconds when introducing 100 nano Molar ssDNA solution to the array. DNAs were diluted in a histidine buffer (114 mM histidine, 142.5 mM 1-Thioglycerol). Due to their negatively charged backbone, strands are attracted to the positive charge on the pad. In Figure 2.15c, the microarray is exposed to a solution containing key 1 oligos and green fluorescently-labeled reporters. Passive hybridization to the immobilized lock oligos (no charge is applied to the pads) is seen. In fact, each key strand complementary to the immobilized oligos has a tail sequence which is complementary to a reporter sequence (5'-CTCAATGTTTCGGACTCAG-GREEN FLUOROPHORE -3')⁸⁴. The reporter sequence containing a green fluorophore with absorption at 526nm and emission at 549 nm reveals the high fluorescence signal from that particular pad shows correct hybridization⁸⁴. In Figure 2.15d the microarray is heated to an oligo-specific temperature and washed with a high salt buffer (50mM sodium phosphate and 500mM sodium chloride pH7.0). The mis-hybridized oligos as well as unbound DNAs are washed away, and the bound DNA complexes remain. Afterwards the fluorescent signals are measured from each pad to verify correct hybridization. During the detection via CCD camera, matched sequences

provide a high signal, and the mismatched sequence is removed after temperature-controlled washing cycles. Finally there is no considerable signal (10 to 30 times less) coming from the mismatched pads. This method is employed for each single stranded DNA, and 24 different strands are immobilized one at a time onto designated pads. One key oligos' selectivity against 24 lock oligos is investigated, and this experiment is carried for all 24 key sequences to see the selectivity of the designed DNA sequences. Table 2.1 shows the lock sequences used during the experiment.

Lock # 1:	5'-7CGTCGCGC-3'	Lock # 13:	5'-7AGCGCCCG-3'
Lock # 2:	5'-7GCGGGGCA-3'	Lock # 14:	5'-7GCGAGCGC-3'
Lock # 3:	5'-7CGCCCGCC-3'	Lock # 15:	5'-7CGCGGTGC-3'
Lock # 4:	5'-7ACGGCGGG-3'	Lock # 16:	5'-7CGCACGGC-3'
Lock # 5:	5'-7GGCCGCCA-3'	Lock # 17:	5'-7CCCCCGGC-3'
Lock # 6:	5'-7CCGGCCCC-3'	Lock # 18:	5'-7CGCGACGC-3'
Lock # 7:	5'-7CCCGGCCC-3'	Lock # 19:	5'-7CGTGCCGC-3'
Lock # 8:	5'-7CGGCCCCC-3'	Lock # 20:	5'-7CGGTGCGC-3'
Lock # 9:	5'-7GGCGCGTG-3'	Lock # 21:	5'-7ACGCCCGG-3'
Lock # 10:	5'-7CGCGTGGC-3'	Lock # 22:	5'-7CGGGGGGC-3'
Lock # 11:	5'-7CCCCGGCG-3'	Lock # 23:	5'-7GTGCGCCG-3'
Lock # 12:	5'-7ACGCGGGC-3'	Lock # 24:	5'-7CGCGCAGC-3'

Table 2.1: Lock Sequences used in the experiment: 7 Symbolizes biotin in the table. Key sequences are complementary to corresponding locks.

Washing Protocol used during Hybridization

The following temperature-controlled washing protocol is pursued. The temperature of the array is raised to 60C and held for 1 minute. Next, the temperature is lowered at each 30 seconds 1C until it reaches 30C. At this point, the microarray is washed with a high-salt buffer (50mM sodium phosphate and 500mM sodium chloride, pH7.0). This method removes any weak secondary structures in the immobilized strands as well as in the complementary oligos, which may keep them from forming Watson/Crick complexes⁸⁴. Moreover, it prevents unwanted DNA/DNA interactions that may occur between partially complementary sequences, such as between two complementary oligos⁸⁴. Then microarray is brought to 24C, and the fluorescence signals are measured from each pad and photographed with a CCD camera. This is the detection system to learn the outcome of hybridization. This temperature-controlled washing and picture-taking steps are repeated until a robust distinguishable signal is obtained from the pads. The imaging system has an excitation wavelength of 525 nm and emission wavelength of 553 nm⁸⁴. In addition to the above procedure, thermally-assisted washing steps are very important because the temperature, high-salt buffer used during the wash, and its amount are critical to obtain a robust signal from the pads.

Structure and Fabrication of the Microarray

Nexogen, Inc. has expanded Nanogen's electronic microarray technology that utilizes electric fields to accelerate and manipulate biomolecules such as DNA, RNA, and proteins on a microarray surface⁸⁴. Each test site on the microarray has an underlying

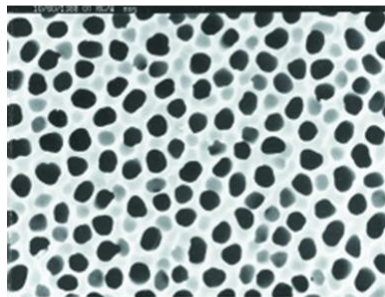
platinum electrode, which can be activated independently due to the integrated CMOS structure⁸¹. The integrated CMOS allows precise control over the voltages and currents, which in turn allow accelerated immobilization of biomolecules onto the individual electrode sites^{85, 86}. This conventional CMOS structure is fabricated in a commercial foundry using 17 level process steps⁸¹. Afterwards, the surface is passivated while growing an insulating layer of silicon dioxide, and vias are etched down to the interconnect metal. Using photolithography and deposition processes, 20 nm titanium and 100 nm platinum are deposited and selectively etched to form electrodes, additional electrical wirings and contacts^{81, 84}. Next, a silicon oxide insulating layer is deposited by plasma-enhanced chemical vapor deposition, finally producing the individual electrodes at each site on the surface of the chip. Each electrode is 50 to 80 μm in diameter depending on the array chosen, and the center-to-center distance between the two electrodes is 150 μm . On the surface of the array, a 1-2 μm thick hydrogel permeation layer containing streptavidin is spin coated and, in later applications, deposited by microreaction molding⁸²⁻⁸⁶. The idea behind using this layer is, first of all, to protect the DNA strands from the undesirable electrochemical effects at the platinum electrode surface during activation of the pads. Second, the permeation layer assists the attachment of biotinylated oligos through biotin and streptavidin binding. This allows the electronic microarrays to concentrate the oligos at the specific pad 1000 times more than low diffusion dependent passive hybridization arrays⁸⁴⁻⁸⁶.

Preparation of Gold-NWs

Template assisted electrochemical deposition is a simple method of synthesizing nanorods and nanowires with a controlled shape and size⁸⁷. Major advantages of this method over other processes are low-temperature operation, less time consumption and wide range of adjustable pore diameters, large number of nanowires production at the end of the process and high reproducibility by control over shape/size^{48, 87}.

Electrolyte solution is formed by dissolving salts, which dissociates to form ions. When electric current is passed through the electrolyte, the ions can be deposited to form solid material. This process is generally referred as electrochemical deposition. This method is used in our lab to fabricate metallic and semiconducting NWs^{47, 48, 87}. In this research electrochemical deposition is used to grow NWs inside an Anodic Aluminum Oxide (AAO) template (Figure 2.16).

Whatman Anodisc 13



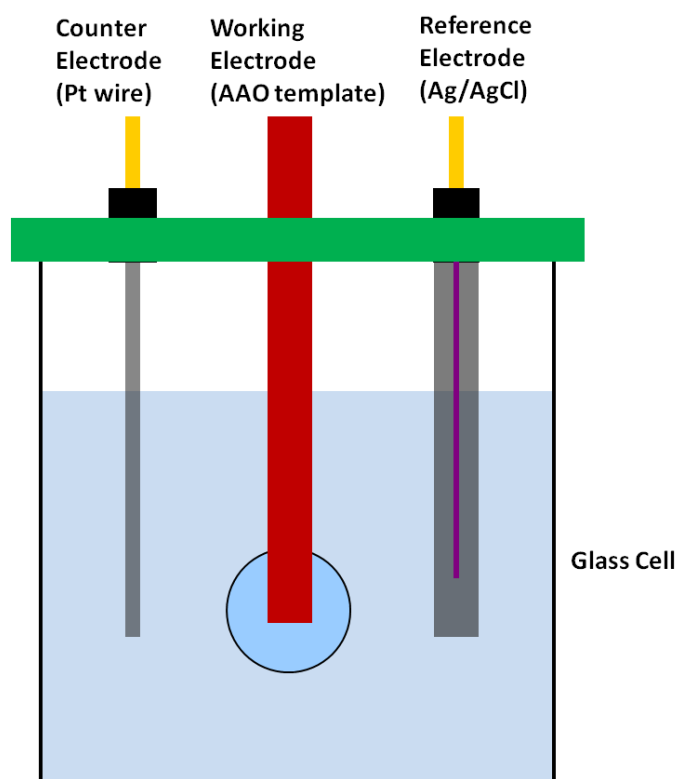
Anodisc pore structure

Figure 2.16: The Anodic Aluminum Oxide (AAO) template (Whatman Anodisc 13) used in the project is purchased from Whatman Ltd.

Electrochemical deposition is carried in a three-electrode electrochemical cell (Figure 2.17). The electrodes are a working electrode (AAO template), a reference electrode (Ag/AgCl) and a counter electrode (platinum spiral rod)⁸⁷. Working electrode is the electrode where the electrochemical deposition to produce NWs takes place. Reference electrode is the electrode whose potential is constant enough so that it can be taken as reference standard and the potential of other electrodes are measured accordingly. Ag/AgCl has 0.197V potential and used in this project⁸⁷. Counter (auxiliary) electrode serves as a source or sink for electrons, so current can be supplied from external circuitry to the electrochemical cell. The auxiliary electrode is a typical platinum wire and it is the surface balances the redox occurring at the working electrode⁸⁷. Therefore, the surface area of the auxiliary electrode must be equal or larger than the working electrode⁸⁷.

For electrochemical deposition to take place in the cell, working electrode should be conducting. Therefore, a thin layer of metal (depending on the desired end result 500nm to 2 μm), Ag or Au is deposited via E-beam evaporator or sputtering machine onto the backside of the template before electrochemical deposition. AAO template is dielectric and due to the thin metal layer the positive charge ions can reach to the bottom of the pores and fill the pores^{48, 87}. The pores are relatively more uniform on the dim side of the template than the shiny side for commercial AAO membranes. Therefore we deposit the backside metal layer to the shiny side to get more uniform NWs⁸⁷. A conductive tape is attached to the back (conducting side) of the template (working electrode) to establish the connection between the potentiostat and working electrode. If the template is used in this

form, deposition will occur at the backside of the template rather than in the pores as desired⁸⁷.



Three electrode electrochemical cell

Figure 2.17: Three electrode electrochemical cell setup for growing nanowires

To avoid this problem, the backside of the template as well as the conducting wire is coated with a non-conductive coating (Microstop stop-off lacquer). In this way, during deposition the only conductive place for ions to get reduced is the conductive layer at the bottom of the pores. The charged ions are introduced to the pores when template is exposed to the electrolyte and current is applied to the conducting layer. The ions reaching to the bottom of the pores accept the electrons and are reduced to the solid form. NWs are electrodeposited from the bottom of the pores. This process ensures mostly

continuous NWs and the time of the deposition determines the length of the NWs⁸⁷.

After deposition the template is dried and put into acetone (CH_3COCH_3) for 40-50 minutes to remove the non-conductive layer at the backside. If desired in the process to remove the metallic conducting layer at the back of the template, template is dipped into 50% nitric acid (HNO_3) solution. To remove the extra acid approximately 5 cycles of rinse with DI (de-ionized) water is required. Finally the solution containing NWs are cleaned by 5 cycles of centrifugation.

The key issue for the electrochemically grown NWs is their crystalline quality. In most cases the NWs are not epitaxially grown; therefore, they are either amorphous or polycrystalline. That limits their applications in devices^{48, 87}. Defects with electrochemical deposition in AAO template are as follows. There is a pore-to-pore variation in nucleation rate due to the grain boundaries or adsorbed impurities at the cathode interface. If some pores are filled rapidly, then the rest of the growth occurs at the surface and closes the unfilled pores in the process. Even small cracks in the template cause deposition to take place at these locations and can be seen from uneven current distribution⁴⁸.

We produced in our lab various NWs with cyclic voltammetry method^{47, 48, 87}. In this method, a potential is applied to the system and the current response is measured. Here the current is faradaic current, which is the current due to the redox reaction^{48, 87}.

Potential is measured between the reference electrode and the working electrode and the current is measured between the working electrode and the counter electrode^{48, 87}.

In this particular experiment, Au NWs were synthesized by electrochemical deposition inside a porous alumina membrane (Whatman Anodisc 13) which has the thickness of 60 μm and cylindrical pores of around 200 nm diameter (Figure 2.18a). Using an electron beam evaporator (Temescal BJD-1800), a thin conducting film of Ag was deposited on one side of the membrane (Figure 2.18b). After coating the backside of the template as well as the conducting wire with a non-conductive coating (Microstop stop-off lacquer), the template was placed in an electrochemical cell set up. The electrodeposition was then carried out by applying a constant current of $2\text{mA}/\text{cm}^2$ for 10 minutes in commercial gold solution (Techni Gold 25ES). During this period the electrolyte solution temperature was kept constant at 55C (Figure 2.18c). By the end of electrodeposition process the grown nanowires were released from the template through dissolution of the alumina template in 3M NaOH for 50 min (Figure 2.18d). Gold nanowires were then cleaned by combination of sonication, centrifugation and rinsing. The final gold nanowires were diluted by deionized water. After this process we obtained Au-NWs with various lengths from 500nm to 2 micron (Figure 2.18e).

Thiols have high affinity to Au surface and form self-assembled monolayers^{47, 61-63}. When we mix the thiolated DNA sequences with the NW suspension and incubate 4 hours, thiolated DNA sequences cover the surface of the NWs.

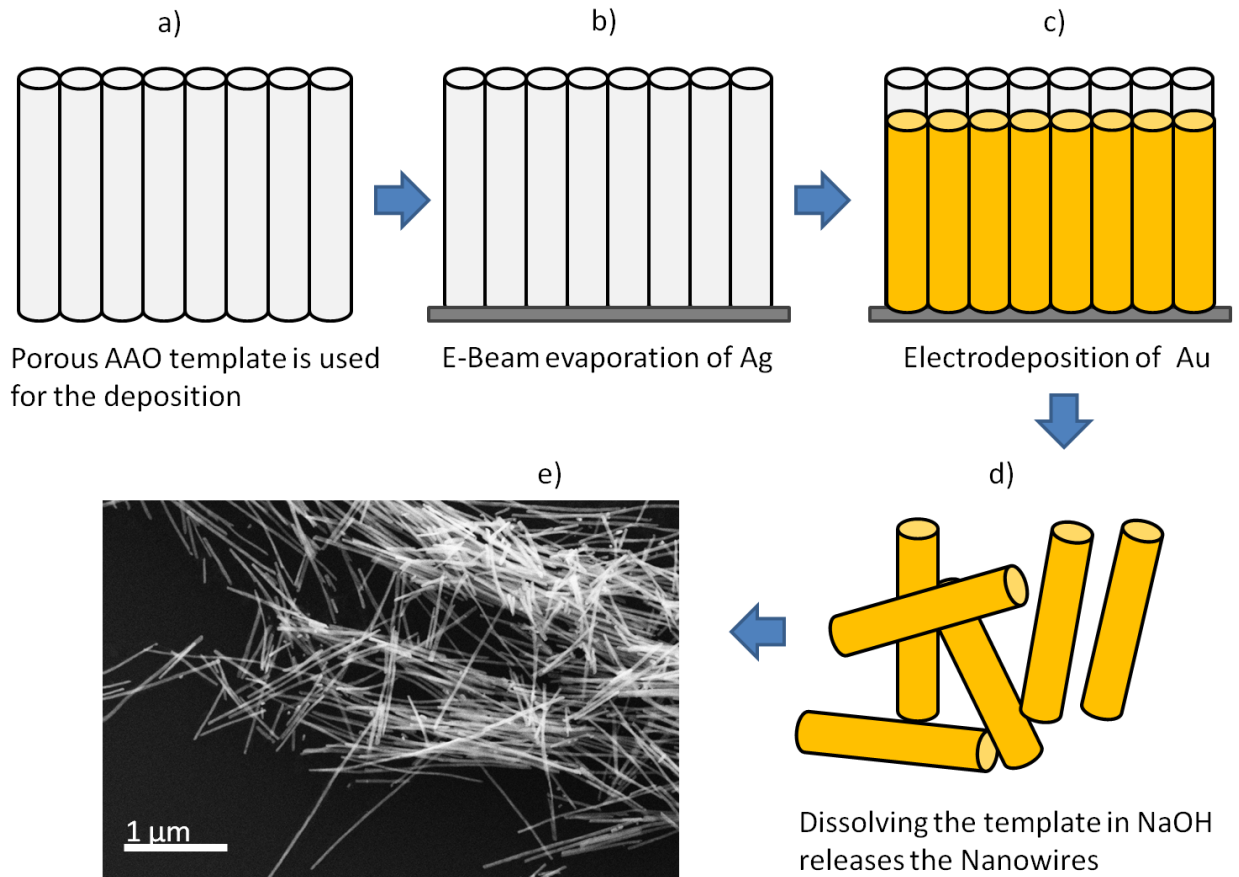


Figure 2.18: Au nanowires fabricated by electrochemical deposition inside Anodic Alumina Membranes. A, b, c and d illustrate the step-by-step deposition process, which is electrochemically filling the 200 nm diameter pores of a porous alumina template and dissolving the template afterwards releasing the NWs. e) is the SEM image of the NWs synthesized.

Preparation of Multi-component NWs

NWs show great potential for applications in nanoelectronics, energy conversion, self-assembly and controlled positioning of NW arrays⁸⁷⁻⁹¹. Electrochemical synthesis can produce multi-component NWs where individual segments are metallic, semiconductor, oxide or alloys with specific properties⁴⁷. Here we will explain the synthesis multi-component NWs which can be assembled end-to-end by DNA.

Au/Ni/Ag nanowires around 200 nm diameter and 10 μm length were fabricated in AAO templates (Anodisc, Whatman) while changing the electrolyte solutions (Figure 2.19a). A thin layer of silver is deposited on one side of an AAO template by E-beam evaporation (Figure 2.19b). A three-electrode cell is used to deposit the Ni segments of nanowires. Ni segments of NWs were deposited at -1.2V for 20 minutes in 0.5 M NiSO_4 , 0.6 M H_3BO_3 solution (Figure 2.19c). After that, Au segment was deposited from commercial solution (Techni Gold 25ES) at $2\text{mA}/\text{cm}^2$ for 15 minutes (Figure 2.19d). The template is dissolved in a 2 M NaOH solution 2 hours (Figure 2.19e) to release the NWs. By using DI water NWs are washed several times and Au/Ni/Ag multi-component NWs are obtained (Figure 2.19f).

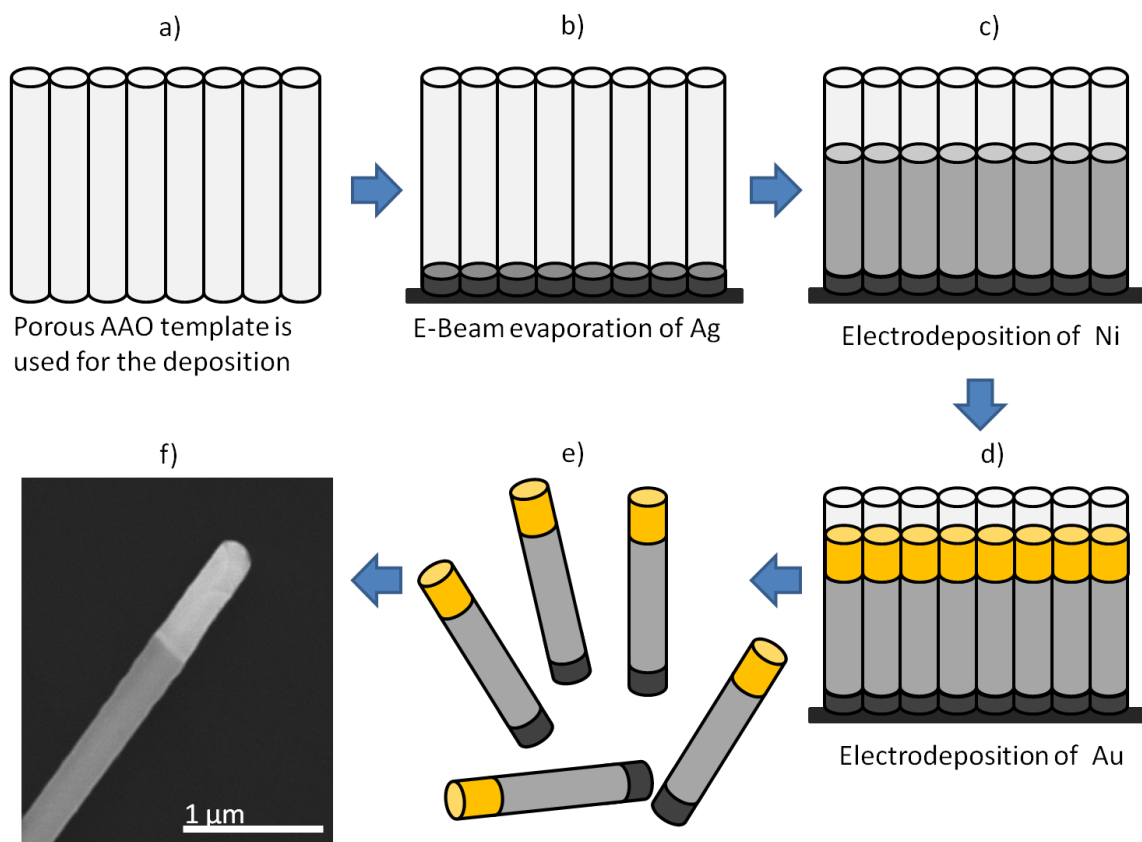


Figure 2.19: A to e illustrates the Au/Ni/Ag multi-component NW synthesis process. f) is the SEM image of the NW synthesized.

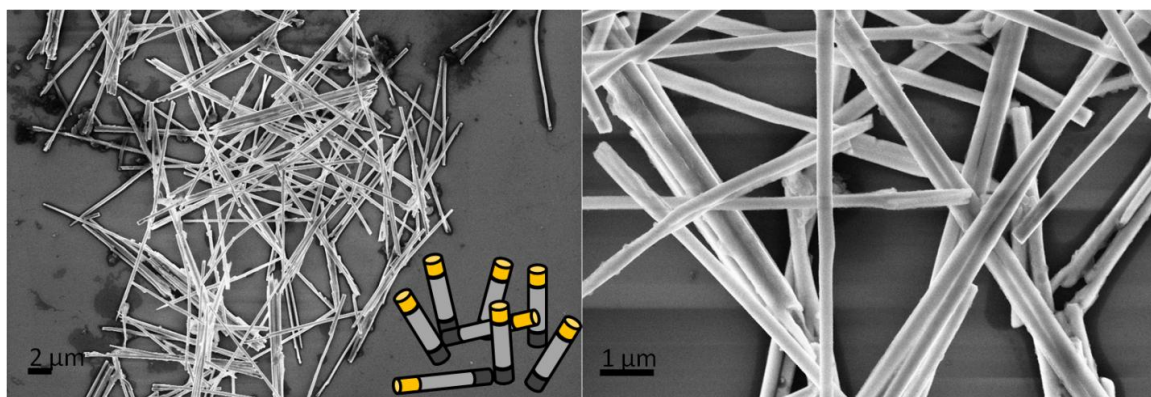


Figure 2.20: SEM images of Au/Ni/Ag multi-component NWs.

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3. Electronic Microarrays in DNA Computing

Abstract: DNA Computing is a rapidly-developing interdisciplinary area which could benefit from more experimental results to solve practical problems with the current biological tools. In this study, we have integrated microelectronics and molecular biology techniques for the storage of information and basic arithmetic operations via DNA. Using 16 different complementary sequences of DNA, we stored 4 bits of information on an electronic microarray and read the data via the fluorescent signal strength coming from the microarray pads. We also showed the possibility of addition and subtraction of quantities of fluorescently tagged DNA determined via their fluorescent signal strength. We conclude that the hybrid technology we employed, based on a matured Si-CMOS platform, has the potential to strengthen the pursuit of DNA Computation as well as finding its own niche applications.

Introduction

DNA computing¹ is a method of parallel computation which uses the selectivity of DNA denaturization to sift through a large space of combinations that would be possible starting with many different strands of DNA. This massive parallelism introduces the possibility that DNA computation may help to solve certain problems more conveniently than could be done using an ordinary silicon computer²⁻⁸. An example would be a computation involving inputs that are already encoded in concentrations of various DNA strands, for example from a genomic library. In addition to this massive parallelism inherent in the biological operations, DNA computing also offers extremely dense

information storage capacity and energy efficiency. Therefore, much work is being done to determine if this potential can be realized in practical use to solve real computational problems that cannot be solved using conventional methods¹⁻¹².

Molecular computing or more specifically DNA computing took off in 1994, when L.M. Adleman in his paper “Molecular Computation of Solutions to Combinatorial Problems” used DNA to solve an example of the Hamiltonian Path Problem, experimentally demonstrating the use of DNA as a computational system¹. Since then, many schemes have been developed using the DNA Computing approach for solving complex problems. One such scheme uses parallel search algorithms in experiments such as solving small scale Hamiltonian Path problems, and the Satisfiability (SAT) problem¹⁻⁸. Another investigates DNA-based nanodevices such as DNA finite automata and DNA fueled Nanomachines^{9,10,13-16,20-22}. A third approach, as followed in Winfree’s Lab in Caltech, implements self-assembling DNA tiles for computation¹³⁻¹⁹, while Lloyd Smith’s group in the University of Wisconsin-Madison performs DNA computations on a solid surface²⁴.

Initial experiments in DNA computation¹⁻⁶ used chemical manipulations carried out in test tubes. This approach required magnetic beads to remove DNA strands from the solution, which are error prone and not easy to scale up for larger problems even if we just consider the different types of beads required for a particular mathematical problem^{23,24,40}. Surface-based DNA computation solves these problems because after

immobilizing the DNA on a solid support, it becomes simpler to analyze DNA between the various steps of the computation²³⁻²⁸.

Our goal in this study is to investigate a reliable platform via feasibility studies for DNA Computing. The Mark-Destroy-Unmark operation was an interesting possibility suggested by Lloyd Smith's group but the disadvantages were the side effects of destroying DNA strands which are in fact the correct solution through the enzymatic reactions during computation and the error prone reading mechanism of the correct solution²³⁻²⁸.

In this study, we use electronic microarrays as our solid surface combining microarray positioning ability with optical readout²⁹. Our system is independent of enzymatic reaction efficiency, does not destroy the DNA sequences and reads the output directly from the surface with a CCD camera. We show that information can be stored and manipulated via the fluorescent signal strength of the hybridized complementary DNA strands using an electronic microarray.

Experimental details

In conventional microarrays, individual pads cannot be controlled but instead the same process is applied to the entire array. In electronic microarrays current or voltage is applied to the test sites (pads) to assist the rapid and controlled transport of charged molecules to the pads²⁹⁻³⁴. This is called pad activation. The advantages of electrically-assisted transport are: (1) the ability to produce reconfigurable electric fields on the microarray surface that allows the rapid and controlled transport of charged molecules to

any pad; (2) the ability to carry out pad selective DNA addressing and hybridization; the ability to significantly increase DNA hybridization rate by concentration of immobilized DNA at the pads; and (3) the ability to use electronic stringency to improve hybridization specificity³⁴⁻³⁷.

In this study, we explore the possibility of DNA based computations on electronic microarray platforms. To illustrate this approach we use DNA to store information and to represent and manipulate numbers.

In the first part of this study, we show that engineered DNA sequences that are labeled with fluorophore can be used for storing information. We wanted to store letters “U”, “C” and “R” to show that user defined information, which is the monogram “UCR” here, can be stored in 3 vials of DNA solution and reconstructed using an electronic microarray via the fluorescent signal strength of the complementary pairs. The idea is that complementary hybridization such that a pair of oligos fluoresces on a microarray pad represents a white pixel or “1”; conversely the absence of hybridization on a microarray pad represents a black pixel or “0” with no fluorescent signal. In order to immobilize the oligos on the electronic microarray pads, we applied a 350 nano Ampere constant current on each pad for 40 seconds when we introduce the 100 nano Molar oligo solution to the array where oligos were diluted in a histidine buffer (114 mM histidine, 142.5 mM 1-Thioglycerol). Due to their negative charged backbone, DNA strands will go to the positive charge on the pad. The pads are made of platinum on silicon and covered with a hydrogel permeation layer that contains streptavidin spin-coated onto the surface. The

permeation layer serves to separate the biological materials from the electrodes and allows 5' biotinylated DNA samples to bind to the pad. We removed the unattached DNA by washing with histidine buffer and we repeat this procedure for each sample^{31, 43-44}. Using this method, we immobilized 16 different oligo sequences independently via application of constant current on a different pad.

A: 5'-7CGTCGCGC-3'
A ^C : 5'-CTGAGTCCGAACATTGAGGCGCGACG-3'
B: 5'-7GCGGGGCA-3'
B ^C : 5'-CTGAGTCCGAACATTGAGTGCCCCGC-3'
C: 5'-7CGCCCGCC-3'
C ^C : 5'-CTGAGTCCGAACATTGAGGGCGGGCG-3'
D: 5'-7ACGGCGGG-3'
D ^C : 5'-CTGAGTCCGAACATTGAGCCCGCCGT-3'
E: 5'-7GGCCGCCA-3'
E ^C : 5'-CTGAGTCCGAACATTGAGTGGCGGCC-3'
F: 5'-7CCGGCCCC-3'
F ^C : 5'-CTGAGTCCGAACATTGAGGGGGCCGG-3'
G: 5'-7CCCGGCCC-3'
G ^C : 5'-CTGAGTCCGAACATTGAGGGGCCGGG-3'
H: 5'-7CGGCCCCC-3'
H ^C : 5'-CTGAGTCCGAACATTGAGGGGGGCCG-3'
I: 5'-7GGCGCGTG-3'
I ^C : 5'-CTGAGTCCGAACATTGAGCACGCGCC-3'
J: 5'-7CGCGTGGC-3'
J ^C : 5'-CTGAGTCCGAACATTGAGGCCACGCG-3'
K: 5'-7CCCCGGCG-3'
K ^C : 5'-CTGAGTCCGAACATTGAGCGCCGGGG-3'
L: 5'-7ACGCGGGC-3'

L ^C : 5'-CTGAGTCCGAACATTGAG- GCCCGCGT-3'
M: 5'-7AGCGCCCG-3'
M ^C : 5'-CTGAGTCCGAACATTGAGCGGGCGCT-3'
N: 5'-7GCGAGCGC-3'
N ^C : 5'-CTGAGTCCGAACATTGAGGCGCTCGC-3'
O: 5'-7CGCGGTGC-3'
O ^C : 5'-CTGAGTCCGAACATTGAGGCACCGCG-3'
P: 5'-7CGCACGGC-3'
P ^C : 5'-CTGAGTCCGAACATTGAGGCCGTGCG-3'

Table 3.1: Sequences used in the experiment (7 symbolizes biotin in the table. Biotin is used to immobilize that particular sequence to the desired pad.)

Each complementary strand to the immobilized oligo has a tail sequence which is complementary to a reporter sequence (5'-CTCAATGTTTCGGACTCAG-GREEN FLUOROPHORE -3'). The reporter sequence containing a green fluorophore with absorption at 526nm and emission at 549 nm gives us the “1” signal from that particular pad. The fluorescent signal is obtained from the reporter probes hybridized to the complementary oligos which hybridizes with the immobilized DNA strands and signal proportional to the concentration of complementary DNA strands is measured (Figure 3.1).

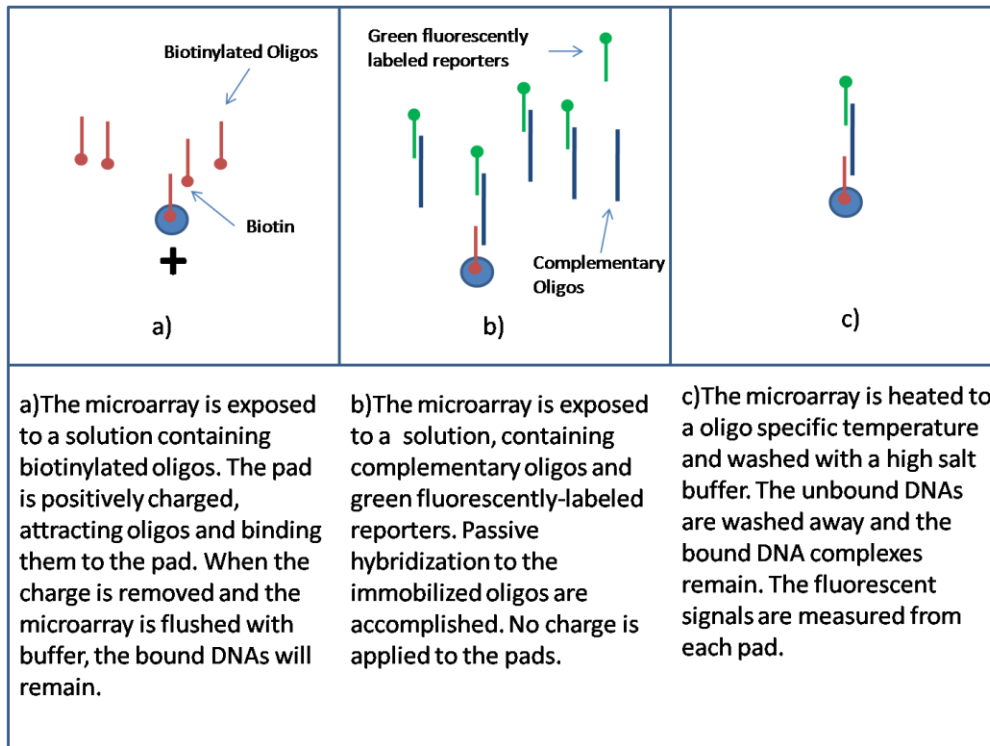


Figure 3.1: Working mechanism of the electronic microarray system

The Structure and Fabrication of the Electronic Microarray

The substrate of the microarray is silicon, over which an insulating layer of silicon dioxide is grown. Using photolithography and deposition processes, 20 nm titanium and 100 nm platinum are deposited and selectively etched to form electrodes, additional electrical wirings and contacts. Next, a silicon oxide insulating layer is deposited by plasma-enhanced chemical vapor deposition. Each electrode is 100 μm in diameter and the distance between the two electrodes is 150 μm . On the surface of the array, a 10 μm thick hydrogel permeation layer containing streptavidin is spin coated or, in later applications, deposited by microreaction molding^{29-34, 42-44}. The idea behind using this layer is, first of all, protecting the DNA strands from the undesirable electrochemical

effects at the platinum electrode surface during activation of the pads. Secondly, the permeation layer assists the attachment of biotinylated oligos through biotin and streptavidin binding³⁵⁻³⁸. This allows the electronic microarrays to concentrate the oligos at the specific pad 1000 times more than low diffusion dependant passive hybridization arrays³⁴.

Hybridization and Washing Protocols

During the hybridization of the oligos the following approach is used. As soon as the solution containing the fluorophore and the complementary oligo sequence is introduced to the microarray, the temperature is increased. This method removes any weak secondary structures in the immobilized oligos as well as in the complementary oligos which may keep them from forming Watson/Crick complexes. Moreover, it prevents unwanted DNA/DNA interactions that may occur between partially complementary sequences, such as between two complementary oligos. The parameters we used here during hybridization are as follows: We start with raising the temperature to 60C for 1 minute. Following the 1 minute, the temperature of the microarray is decreased in steps, lowering the temperature at each 30 seconds 2C until it reaches 30C. At this point, the microarray is washed with a high salt buffer (50mM sodium phosphate and 500mM sodium chloride pH7.0). The microarray is then brought to 24C and the green fluorescence signals are measured. The imaging system has an excitation wavelength of 525nm and emission wavelength of 553 nm. In addition to the above procedure, thermally-assisted washing steps are very important. The wash temperature, the wash

buffer and the wash buffer amount is critical to obtain a robust signal from the pad. In the first part of the study we used 3 times 400 micro liter high salt buffer to achieve a reliable image. In the addition and subtraction operations of the second part of the study, we used low salt buffer in addition to high salt buffer. Low salt buffer is basically half the concentration of the high salt buffer.

Results and discussion

We labeled the 16 immobilized DNA strands on the microarray pads as A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P. Table 3.2 shows the 16 complementary sequences we introduced to the pads to obtain each letter of our monogram “UCR”. For example, we poured onto the array complementary pairs: A^C, D^C, E^C, H^C, I^C, L^C, M^C, N^C, O^C, P^C to obtain letter U (Figure 3.2).

Letter We Want	Complementary Oligos Required to send to the pads
Letter U	A ^C , D ^C , E ^C , H ^C , I ^C , L ^C , M ^C , N ^C , O ^C , P ^C
Letter C	A ^C , B ^C , C ^C , D ^C , E ^C , I ^C , M ^C , N ^C , O ^C , P ^C
Letter R	A ^C , B ^C , E ^C , G ^C , I ^C , J ^C , M ^C , O ^C

Table 3.2: Letters and the complementary oligos required to send to the pads to obtain them

To make a letter, we filled a vial with complementary oligos and reporters mixed in high salt buffer (50mM sodium phosphate and 500mM sodium chloride pH7.0) and water, where the solution reached 100 micro-liters. The complementary oligo concentration in each vial was approximately 100 nM while reporter concentration was around 250 nM. After hybridization of oligos, the microarray is washed with high salt buffer at elevated

temperatures ranging between 30C and 40C. These washing cycles at different temperatures will hold the matched pairs, force the mismatched pairs to dissociate and remove the unbound complementary pairs and reporters. After washing the microarray, we imaged the microarray with a CCD camera to obtain the letters in Figure 3.3 one by one. The advantage of the electronic hybridization as explained above is its accuracy and speed. It takes around 100 seconds compared to the 1-2 hours required for passive hybridization and provides much more accurate and specific hybridization due to the optimal hybridization conditions on the 100 micron size pads³⁴. This procedure demonstrates that human-defined information can be stored in the content of a vial and can be reconstructed with an electronic microarray.

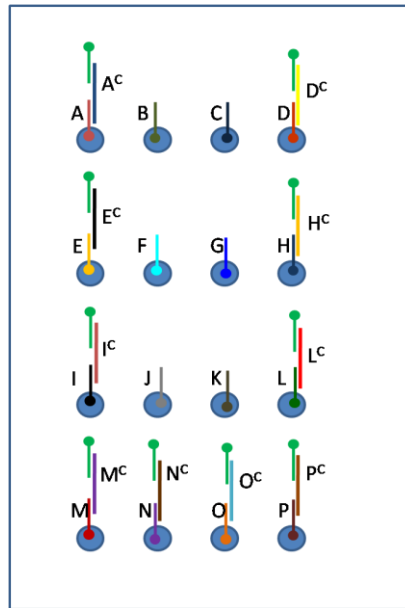


Figure 3. 2: Example showing how to obtain letter “U” Schematically.

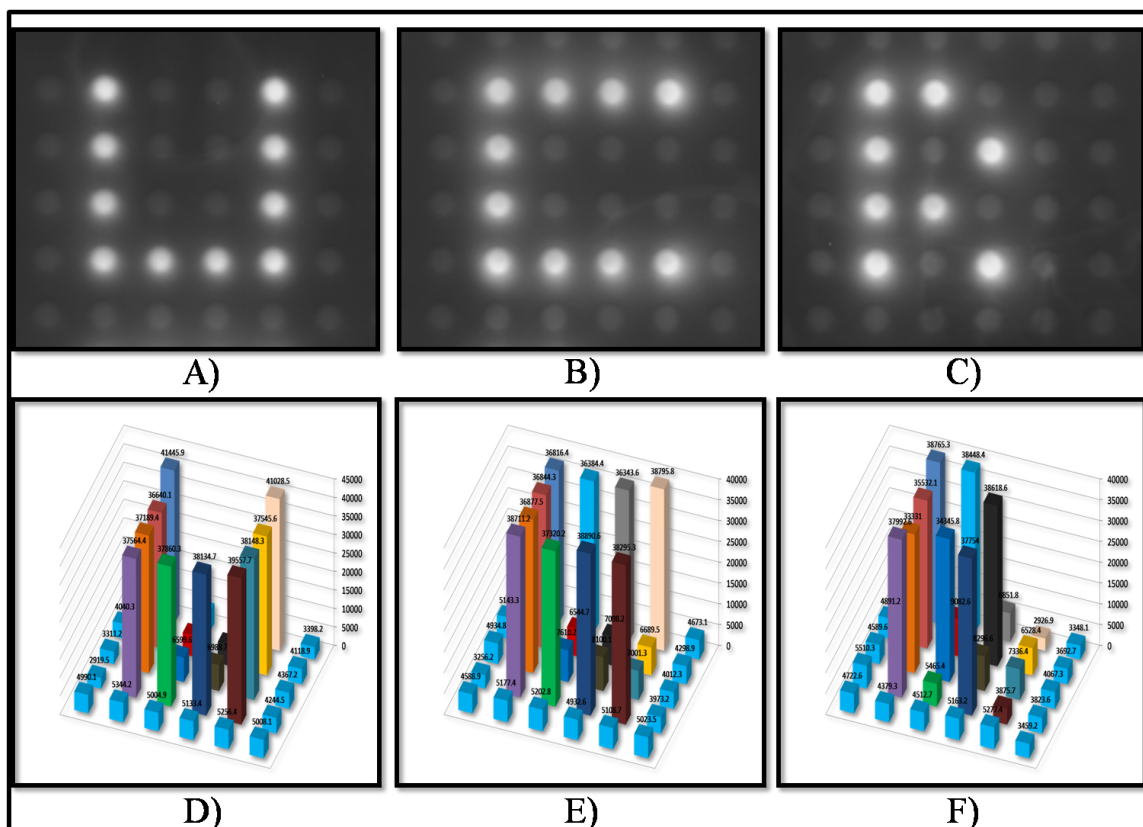


Figure 3.3: The letters UCR are obtained. (A), (B) and (C) are the pictures acquired with the CCD camera and (D), (E) and (F) are the corresponding signals.

In the second part of this study, we adjusted the fluorescent signal from the microarray pads so that it can cover a range of signal strength values which may be interpreted as representing different numbers from 0 to 9. After immobilizing one DNA strand on a microarray pad, we introduced different concentrations of the complementary oligos ranging from 25 nano Molar to 100 nano Molar, with their reporters. This way we obtained fluorescent signals covering a wide span from 1,000 to 65,000 arbitrary intensity units to represent numbers. Table 3.3 shows the range of signals we defined as corresponding to the numbers 0-9. We assigned double the fluorescent signal strength range for the numbers 0, 1, and 2 for convenience so that the images of the lower intensity spots could be discerned visually in Figures 3.4 and 3.5.

Fluorescent Signal Strength in r.f.u.	Corresponding Number
65,000 – 60,000	9
60,000 – 55,000	8
55,000 – 50,000	7
50,000 – 45,000	6
45,000 – 40,000	5
40,000 – 35,000	4
35,000 – 30,000	3
30,000 – 20,000	2
20,000 – 10,000	1
Below 5,000	0

Table 3.3: The fluorescent signal range obtained from the pads and the corresponding numerical value

The concentrations of the complementary pair solutions were chosen so that the signals coming from the pads were approximately linear. Figure 3.4 shows that the fluorescence intensity is approximately linear with the concentration of oligomer, as expected. This linearity should allow the manipulation of DNA oligomer concentrations to effect basic arithmetic operations.

Furthermore, we showed ability to adjust the fluorescent signal strength of the complementary pairs by implementing various washing cycles at different temperatures. (Figure 3.5) After having a signal from the microarray pad in the desired range, for example the signal representing number 2, we introduced to the array a saturating concentration of the complementary solution which is subsequently reduced by means of washing cycles to any desired level. This process can be repeated on the pad many times

without losing the accuracy of the signal because the immobilized oligos on the pads are not affected by the washing steps we apply due to their biotin streptavidin bonds.

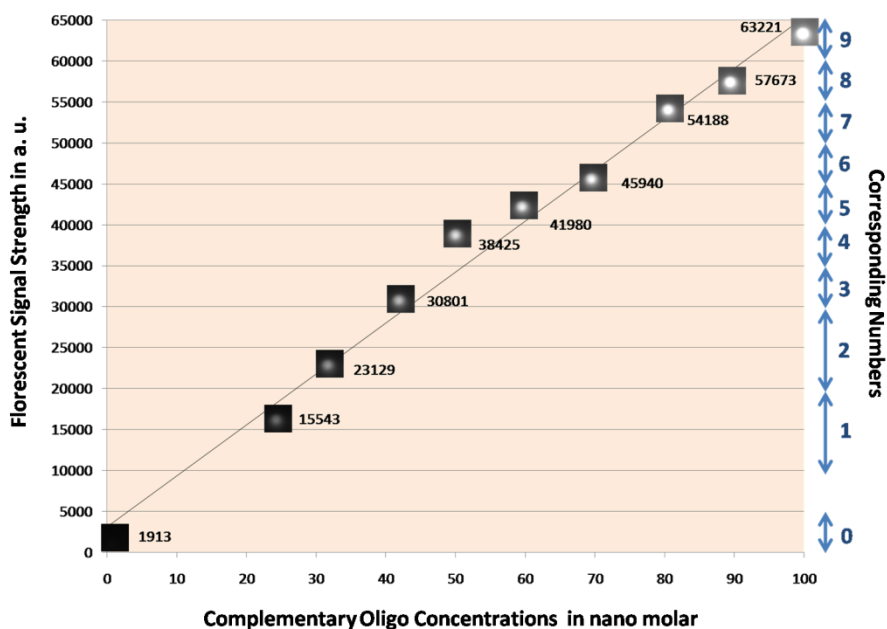


Figure 3.4: Florescent Signal Strength from complementary oligos used to represent numbers

For reduction of the single intensity, only temperature controlled washing cycles are needed. The cycles of increasing or decreasing intensity may be repeated using more than 50 washing cycles on each pad without losing accuracy of the signal at the end of the operation, proving the accuracy, reliability and repeatability of the system. Each number on the x axis of Figure 3.5 is a temperature controlled wash step and explained in the Table 3.4 in detail. This demonstrates that in principle addition and subtraction can be carried out using an electronic microarray platform with accuracies sufficient to resolve at least 10 different intensity levels if one is using assigned numerical values that are linear in the fluorescent signal strength.

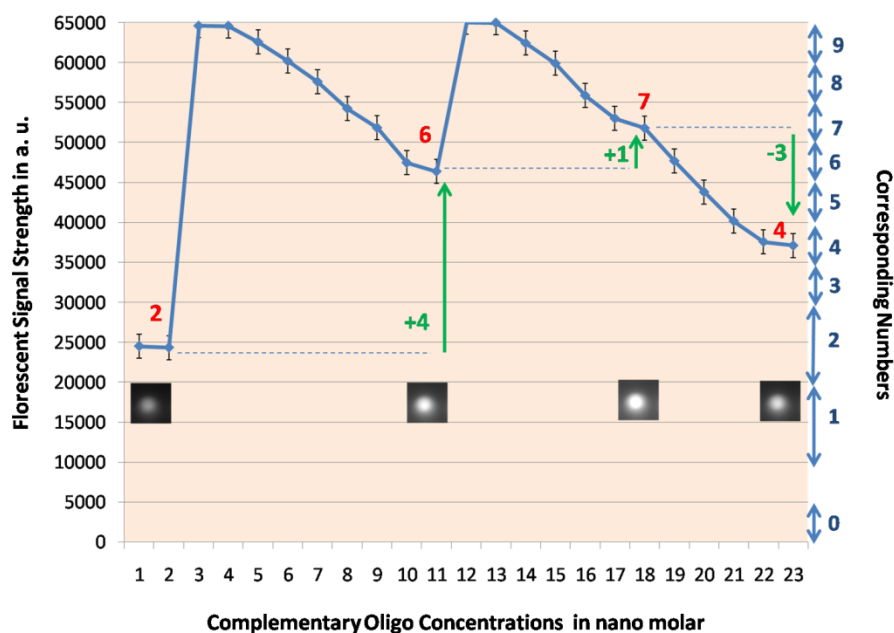


Figure 3.5: Addition and subtraction operations are demonstrated using the numerical values set by the strength of fluorescence

Step	Executed Protocol
1	Low concentration solution of the complementary oligos with their reporters (32 nano Molar in order to get a signal around to 2) is introduced to the microarray and array is washed at 27C with High Salt Buffer (HSB).
2	Array is washed at 27C with Low Salt Buffer (LSB). Signal is stable and represents the number 2.
3	High concentration solution of the complementary oligos with their reporters (100 nano Molar) is introduced to the array and it is washed at 27C with HSB.
4	Microarray is washed at 27C with HSB.
5	Microarray is washed 2 times at 30C and 32C with HSB.
6	Microarray is washed 3 times at 32C, 33C and 34C with HSB.
7	Microarray is washed 3 times at 34C, 35C and 36C with HSB.
8	Microarray is washed 3 times at 36C, 36C and 37C with HSB.

9	Microarray is washed 2 times at 37C with HSB.
10	Microarray is washed 2 times at 38C with HSB.
11	Microarray is washed at 38C with LSB. Signal is stable and represents the number 6. First addition operation is completed $2 + 4$ obtaining 6. Achieving the desired number is accomplished via the carefully controlled washing processes described above.
12	To show more than 1 operation on a pad (in fact, we can make 50 addition and subtraction operations on each pad), we will add 1 to 6. First, high concentration solution of the complementary oligos with their reporters (100 nano Molar) are introduced to the microarray, then the microarray is washed at 27C with HSB.
13	Microarray is washed at 27C with HSB.
14	Microarray is washed 2 times at 30C and 32C with HSB.
15	Microarray is washed 3 times at 32C, 33C and 34C with HSB.
16	Microarray is washed 3 times at 34C, 35C and 36C with HSB.
17	Microarray is washed 3 times at 36C, 36C and 37C with HSB.
18	Microarray is washed at 37C with LSB. Signal is stable and represents the number 7. Second addition operation is complete $6 + 1 = 7$ is achieved.
19	The third operation on this pad is subtraction. In subtraction, only temperature controlled washing is performed. Microarray is washed 2 times at 37C and 38C with HSB.
20	Microarray is washed 2 times at 38C and 39C with HSB.
21	Microarray is washed 2 times at 39C with HSB.
22	Microarray is washed at 40C with HSB.
23	Microarray is washed at 40C with LSB. Signal is stable and represents the number 4. $7 - 3 = 4$ is performed.

Table 3. 4: Explanation of the temperature controlled washing steps of Figure 3.5 X axis:

Conclusion

In the search of finding better experimental methods and tools for DNA Computation, we propose the use of electronically-accessible microarrays, where this technology connects each test site on the array with an electrode, as a valuable tool for storing and manipulating information. Gel electrophoresis is the most common method in DNA Computing so far²³. As shown above, electronic microarray technology can offer reduction in the consumption of DNA, as well as miniaturization of the system with its fast, reliable and repeatable results. Even in this very early stage, DNA Computation is showing promise to exploit the inherent massive parallelism of DNA operations. Using DNA or many other biological molecules, which have evolved over billions of years, in the computation shows the great potential of these molecules and their further study. DNA Computation methods will not replace silicon computers in the near future but can be valuable complementing tools in their own niche markets with their own specific applications such as evolutionary computations³⁴⁻³⁶. Therefore in the advancement of this field, practical and easy to implement ideas where the system is already developed are vital. We showed in this paper that the electronic microarray technology is one of these novel ideas. We conclude that an electronic microarray based technology for manipulation, concentration and hybridization of DNA on the chip array is a useful and reliable tool for future applications.

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4. Electric Field Accelerated Transport of DNA Molecules for faster DNA Computing

Abstract: DNA Computing is a promising concept for accelerating computation of large amounts of data. In this study, microelectronics and molecular biology techniques have been integrated to introduce a new DNA Computing platform that uses semiconductor processing and CMOS (Complementary Metal-Oxide-Semiconductor) technology for accelerated transport of DNA molecules. This hybridization approach leads to faster computation. To demonstrate the capabilities of the platform and experimentally prove its reliability and stability, 2 Satisfiability (SAT) problems were solved via 2 different methods. It is notable that this approach eliminated the need for PCR (Polymerase Chain Reaction) and enzymes, and as a result error rates and cost are decreased. Overall, this technique shows some significant advantages over previous experimental techniques such as short operating time, reusable surface and simple experimental steps.

Introduction

DNA computing¹ is a novel and fascinating development at the intersection of molecular biology, biochemistry and computer science. The central vision of DNA Computing is processing information and performing computations using the reactions of individual molecules with molecular biology laboratory procedures. Additionally, the development of DNA Computing transfers knowledge between information processing, nanotechnology, and biology with the potential of changing our understanding of the theory and practice of computing.^{2,3} The enormous parallelism (10^{14} molecules at a time),

extraordinary energy efficiency (2×10^{19} operations per joule), and ultra-dense information storage (1 gram of DNA can hold about 10^{14} MB of data, equivalent to approximately 140 trillion CDs) inherent in DNA gathered considerable attention with the hope that someday molecular computers can provide the solution to particular problems which resisted to conventional methods.⁴ This new computational paradigm, DNA Computing, emerged in 1994, when Adleman solved the Hamiltonian Path Problem using the selectivity of DNA denaturization. Adleman showed the possibility that DNA computers can solve hard problems such as NP (Nondeterministic polynomial time) complete problems with their massively parallel computation capabilities.¹ Subsequently, Lipton solved the SAT problem with DNA.⁵ A SAT problem is a decision problem written in Boolean expression. The goal in a SAT problem is to determine appropriate assignments of a set of Boolean variables with values of either “true” or “false” such that the output of the whole Boolean formula is true.⁶⁻⁸ In 2000, Liu et al. solved SAT problem using a surface-based DNA computation approach.⁷ In 2002, Braich et al. solved a 20-variable instance of this NP complete SAT problem – the largest yet solved computational problem by non-electronic methods.⁸ Solving SAT problems with DNA became the benchmark in DNA computing for testing the performance of the model and various proposals developed.⁹⁻¹²

The next challenges for DNA Computing to overcome are improvements in control and reliability as well as reduction in cost and error. At that point, surface-based methods offer advantages that can make DNA Computing a candidate for commercialization and applications to difficult computational problems.

There are 4 advantages to using surfaces rather than solutions for DNA computing:

- 1) Reactions are easier on a surface than in solution. In solutions, the DNA reactions take place on a surface, and the remaining material is washed away after operation to remove the unwanted reaction products.
- 2) The transfer of the strands from tube to tube after each step is eliminated in the surface approach. This minimizes loss of DNA and, therefore, minimizes error.
- 3) Immobilized strands will not interact with each other. This reduces interference and false signals.
- 4) DNA computing can benefit from the tools in silicon processing. Tools designed for integrated-circuit fabrication such as patterning and etching can be used in DNA computation on a surface. Parallel processing of DNA and controllability of surfaces can be combined.¹⁵

Since 2000, Smith⁷ and co-workers have shown the significant role of surface science in harnessing the parallel nature of DNA Computation. Their method on the foundation consists of 6 components:

- 1) Making a set of single strand DNAs where each strand represents a candidate solution.
- 2) Immobilizing these strands to the surface in an unaddressed fashion.
- 3) Hybridizing with “mark” operation in each cycle of the computing a subset of surface bound strands with their complementary strands.

- 4) Eliminating with ‘destroy’ operation the non-hybridized strands on the surface via *E. coli* exonuclease I enzyme.
- 5) Preparing the surface for the next computing operation with “unmark” operation which is removing the hybridized complementary strands from the surface bound strands.
- 6) Interpreting the result of the computation via “readout” operation which is PCR followed by hybridization to an addressed array. After repeating these “mark”, “destroy” and “unmark” operations, only the strands which are solutions to the problem remain on the surface; all the other DNA strands are removed.⁷

Despite the progress of Smith and others over the past several years, DNA Computing still has disadvantages, which should be noted for further improvement. First, in their DNA Computing model, n variables require 2^n unique DNA strands. Second, each “destroy” operation requires enzyme activity, which increases the cost and enzymatic reaction efficiency introduces error to the computation (leaves the strands that should not be removed and vice versa). And third, after computing PCR is required to read the results; this adds another potential error source as well as making the process of computing longer.⁷

To address these shortcomings, researchers have bound DNA to various materials such as plastic¹⁶, glass¹⁷, gold¹⁸ and silicon¹⁹. This chapter presents the first results of DNA Computing in a silicon chip. On a CMOS platform, multiple pads can be created, and the electric field of the desired pad on the surface can be activated selectively. The electric field causes electrophoretic movement of DNA molecules due to DNA’s charged

backbone. The pads are spin-coated with streptavidin-containing permeation layer, and biotinylated oligos are immobilized on the desired pads. This active control of DNA transport to the desired direction reduces the immobilization time of oligos from several hours to 1 minute and promotes fast hybridization reactions through accelerated specific binding and leads to faster computation. Here the unique capabilities of this technology are proven while solving 2 different SAT problems. The parallel processing capability of DNA and the controllability of surfaces through CMOS technology can broaden the horizons of DNA computing.

Results

Variables attached in tandem

In the first project, the system is used to solve a SAT problem where variables (oligos) are attached in tandem. This approach is developed on top of Haoyang Wu's proposed theory.²⁰ Starting with 8 different short DNA strands consisting of 13 or 14 base pairs (Figure 4.1) (named w, x, y and z to represent the variables, and each variable has 0 and 1 state) 16 different combinations are obtained. 16 different strands from S0 (corresponding to w=0, x=0, y=0 and z=0) to S15 (w=1, x=1, y=1 and z=1) were purchased from Sigma Aldrich after the design. Each possible solution of the SAT problem is represented by these DNA sequences. These sequences are immobilized on to a different pad as explained in the experimental section, representing the solution space to be searched for computation. (Figure 4.1 b)

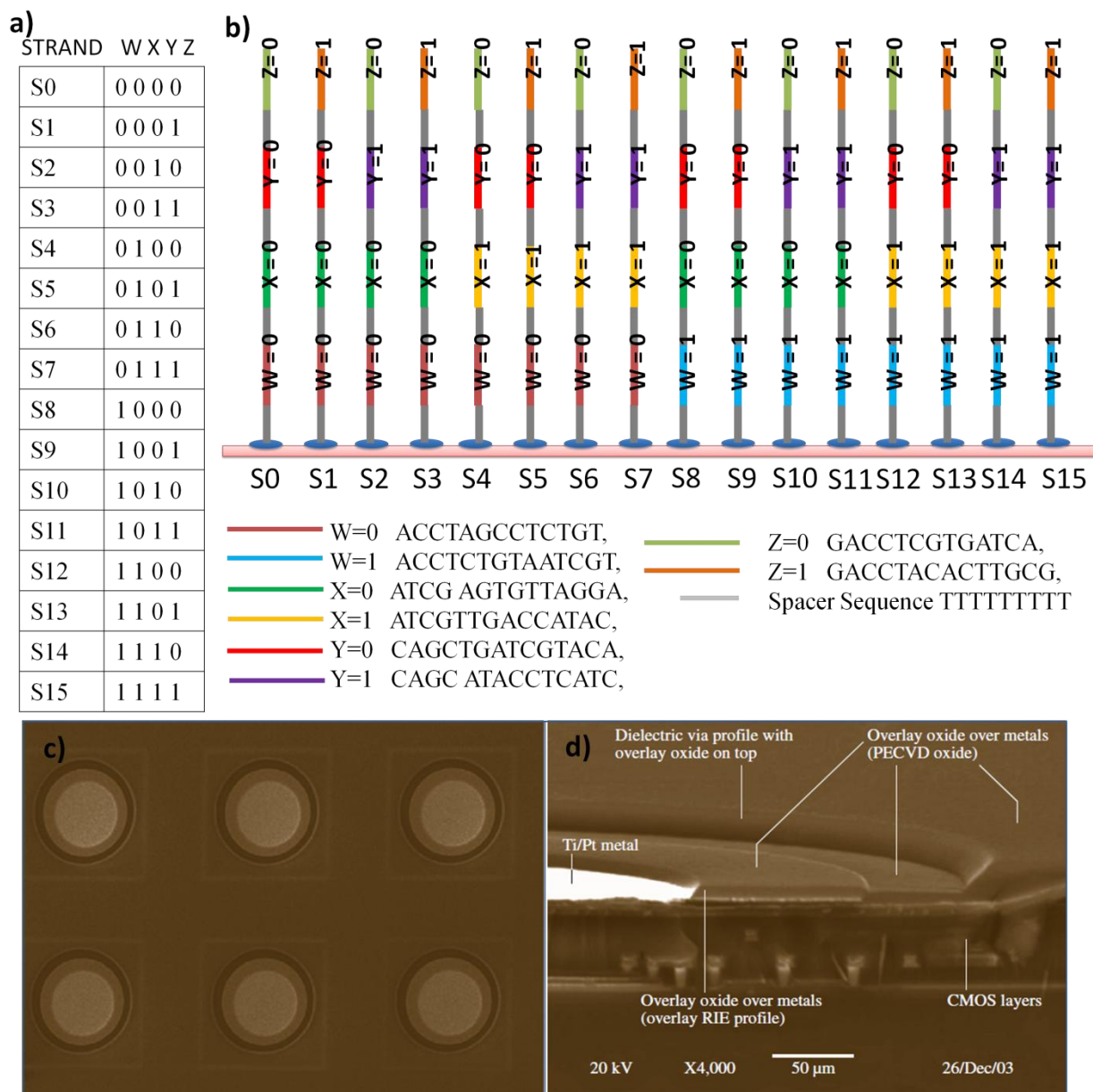


Figure 4.1: a) DNA sequences and the variables they carry. b) Illustration of solution space from S0 to S15 to be searched during computation. Variables and the spacer sequences inserted in between variables can be seen. Each strand is immobilized to the selected pad while applying a constant current to that pad. c) The CMOS chip has an array of 16×25 (400) sites (pads); each electrode is 50 μm in diameter with a 150 μm center-to-center distance. d) Cross-section of an individual microelectrode and the underlying CMOS circuitry.

In the 1st SAT project, the following randomly chosen 4-variable, 6-clause problem was selected.

$$(w \vee x \vee y') \wedge (x \vee y) \wedge (y \vee z) \wedge (w' \vee z) \wedge (y \vee z') \wedge (w' \vee y' \vee z')$$

During the computation step, the corresponding complementary strands for each clause are introduced to the surface, and the response image is taken after controlled washing cycles. For example, for the clause $(w' \vee z)$, the solution composed of oligos complementary to $w=0$ (represented as $C(w=0)$) and oligos complementary to $z=1$ (represented as $C(z=1)$) are sent to the surface without applying any charge on to the pads (Figure 4.2). Each complementary strand, other than the variable that is going to hybridize, needs to hybridize with a reporter sequence for detection. Therefore each complementary strand is ligated to an additional DNA sequence (represented as $C(\text{Reporter})$), which is complementary to reporter sequence. The reporter sequence (5'-CTCAATGTTTCGGACTCAG-Green Fluorophore-3') has a green fluorophore with absorption of 526 nm and emission of 549 nm and is used to indicate the correct hybridization from that particular pad after correct hybridization. When introduced the DNA solution mixture for clause $(w' \vee z)$ to the platform, all satisfying answers of this clause in the solution space (an immobilized strand on the array that can satisfy 1 or 2 parts of this clause) will hybridize. This procedure is named asking the query for clause $(w' \vee z)$ to the solution space. The illustration of the array (Figure 4.2a) and the fluorescent image after the query for clause $(w' \vee z)$ are seen in Figure 4.2b. All pads other than pads 8, 10, 12 and 14 are fluorescing, meaning that only these pads do not

have any variables to satisfy this clause. Therefore, they cannot be a solution to the SAT problem. After each clause, the array is washed extensively to eliminate all the hybridized sequences and prepare for the next query (Figure 4.2c).

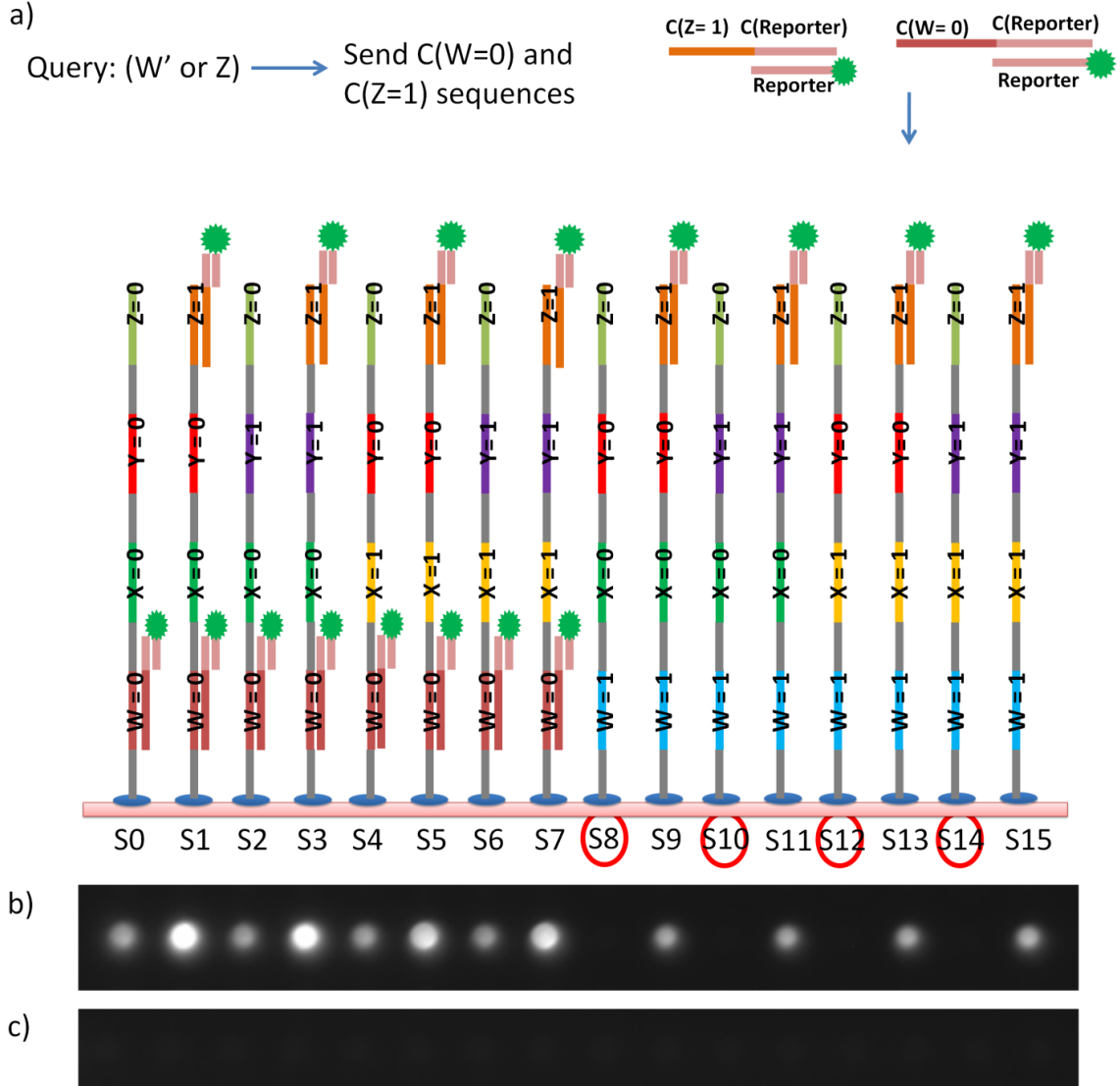


Figure 4.2: Illustration and experimental result of an example query. a) Illustration of asking clause $(w' \vee z)$ to the solution space and the keys elements used. b) After correct hybridization we get fluorescent signal from all pads except pads carrying S8, S10, S12, and S14 strands. This is confirmed with the image taken here c) After obtaining the answer of a query the pads are washed at 60C and hybridized sequences are eliminated preparing the platform for the next query.

In Figure 4.3, the computation steps for the SAT problem is shown starting with the query 1 ($w \vee x \vee y'$) and introducing the complementary strands $w=1$, $x=1$ and $y=0$. Pads 2 and 3 do not have any variables to satisfy this clause as seen via the lack of fluorescence signal coming from these pads. As explained in the experiment section, after recording the answer of a query, the surface is washed at 60C with high salt buffer until all of the hybridized sequences are eliminated. Then the solution space is ready for the next query. Figure 4.3 shows the result after one query at a time has been asked. The answer to the SAT problem can be seen easily by recording the fluorescence images after each clause and putting them on top of one another. The pads that satisfy all the queries, (i.e., the pads which fluoresce after every query) are pads 6 and 7. The corresponding variables on these pads, therefore the answer of the SAT problem, are ($w=0$, $x=1$, $y=1$, $z=0$) and ($w=0$, $x=1$, $y=1$, $z=1$) as seen in Figure 4.3.

As the array is reviewed after each query, the fluorescent signal levels from the pads are also recorded individually. This makes it possible to visualize the solution numerically. In Figures 4a and 4b, the queries and their corresponding fluorescent signal levels are shown. During the computation, the largest mis-hybridized signal was 3400 Relative Fluorescent Units (RFU), compared with the minimum correct positive signal of 29300 RFU. High hybridization specificity can be checked with signal-to-noise(S/N) ratio values during the computation, which was between 13 and 66(in biological processes 5 is acceptable for decision making), reveals the system's reliability to determine the correct sequences.

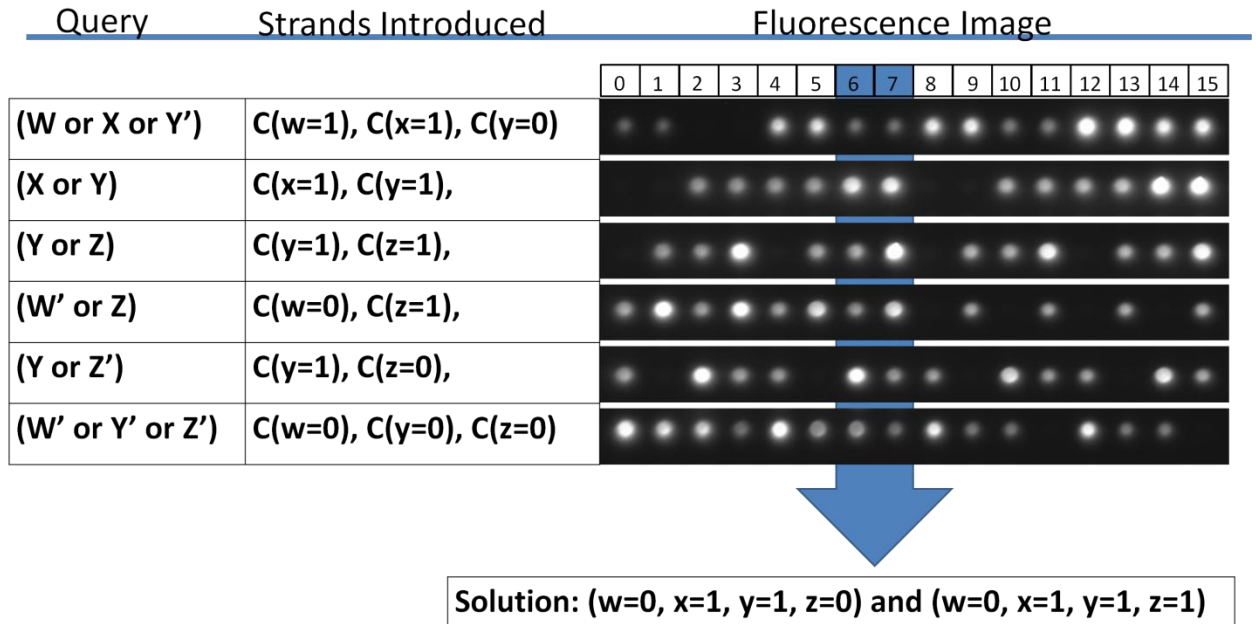


Figure 4.3: Each clause is a query and introduced the solution space. During each query DNA solution composed of complementary strands and reporter sequences according to the content of the query is introduced to the array. After the array is washed and the mis-hybridized pads are eliminated, the resulting image of the array is recorded for each query with a CCD camera. The answer for each query is recorded as black-and-white images, and when these images are lined up the answer to the SAT problem can be seen. The answer satisfies all the queries, which is indicated by the arrow in the figure. These pads are 6 and 7, with their corresponding variables (w=0, x=1, y=1, z=0) and (w=0, x=1, y=1, z=1).

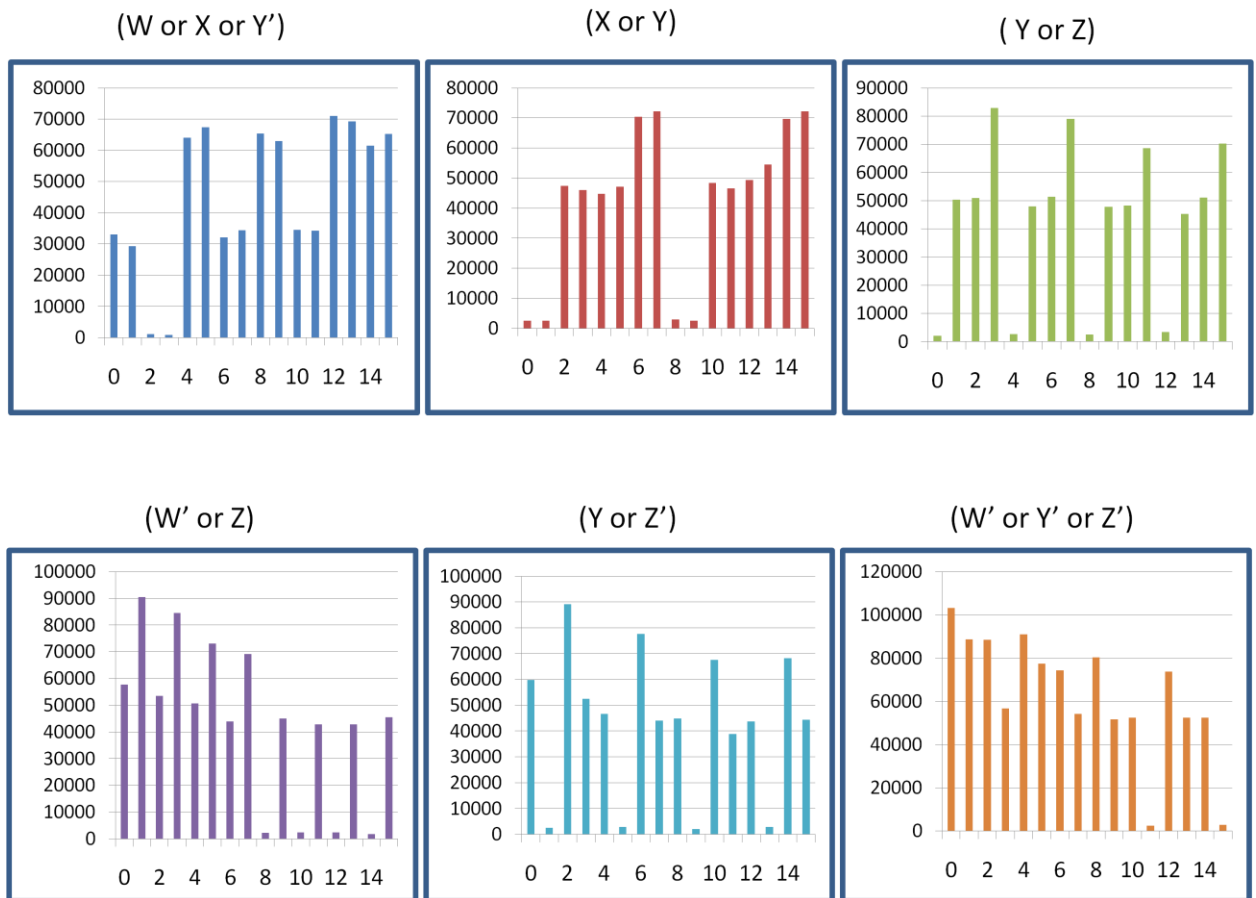


Figure 4.4a: The fluorescent intensity histogram after each query in RFU (Relative Fluorescent Units).

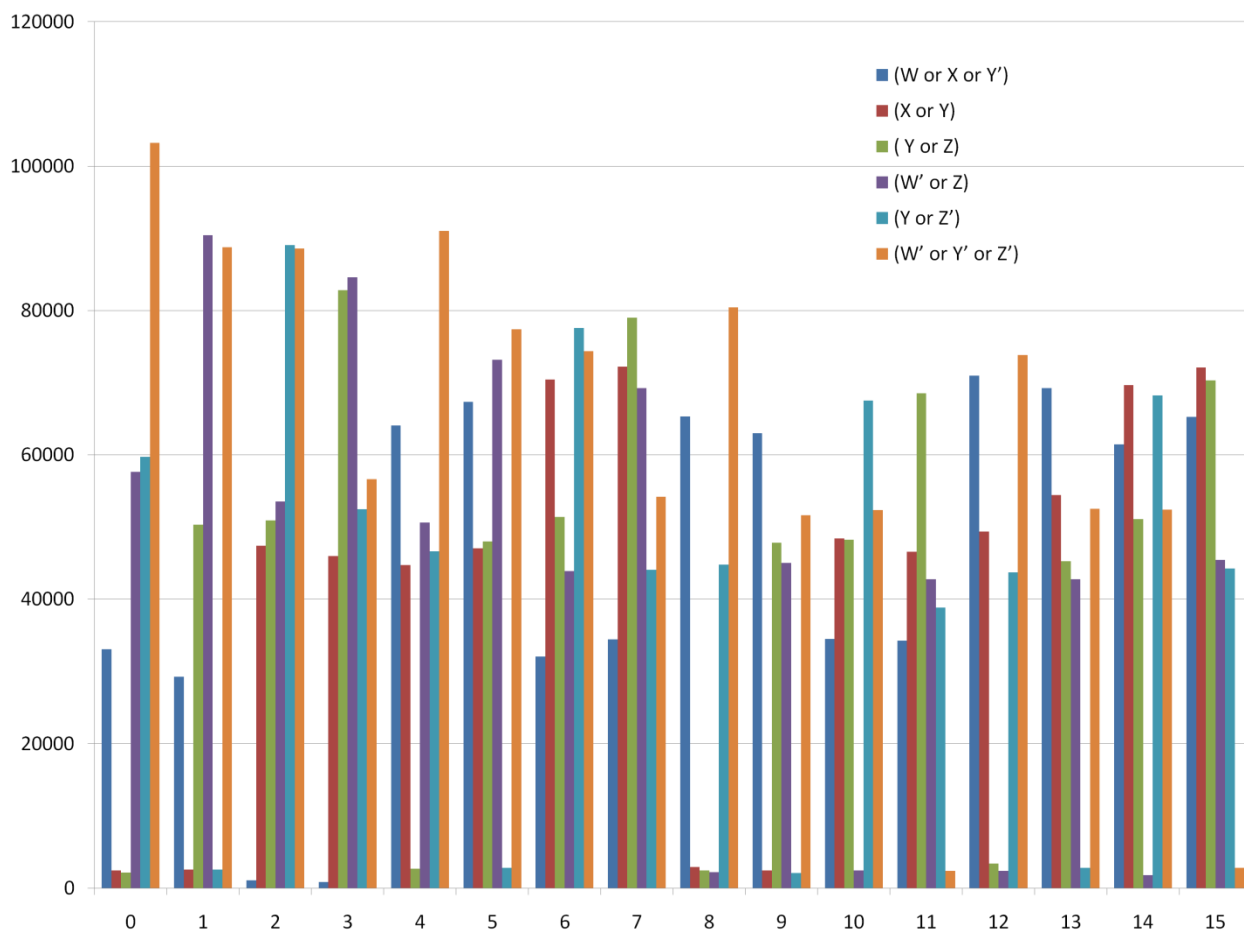


Figure 4.4b: The fluorescent intensity histogram of the SAT Problem where each query's response recorded in RFU (Relative Fluorescent Units). This histogram clearly shows sequences 6 and 7 only have signal levels above 20000 for all the queries, making them the answer of the SAT problem. All other pads have one or more responses below 20000 rfu, therefore fail to satisfy one or more queries.

One variable on a pad

In the second project, the system was tested for its capability to scale up for larger problem. Attaching one variable per pad approach was proposed by Yin et. al. without experimental realization.²¹ In this project this idea is further expanded and realized experimentally. A 5-variable SAT problem was taken starting with 10 different oligos consisting of 13 or 14 base pairs (oligos are named v, w, x, y and z to represent the

variables, and each variable has a 0 and 1 state, making 10 ssDNA in total). Each variable was immobilized in such a way that each column on the platform represents values from 0 to 31 as seen in the Figure 4.5. For example, the 1st column represents value 0 because it has immobilized variables $v=0$, $w=0$, $x=0$, $y=0$ and $z=0$. The last column represents value 31 because it has immobilized variables $v=1$, $w=1$, $x=1$, $y=1$ and $z=1$.

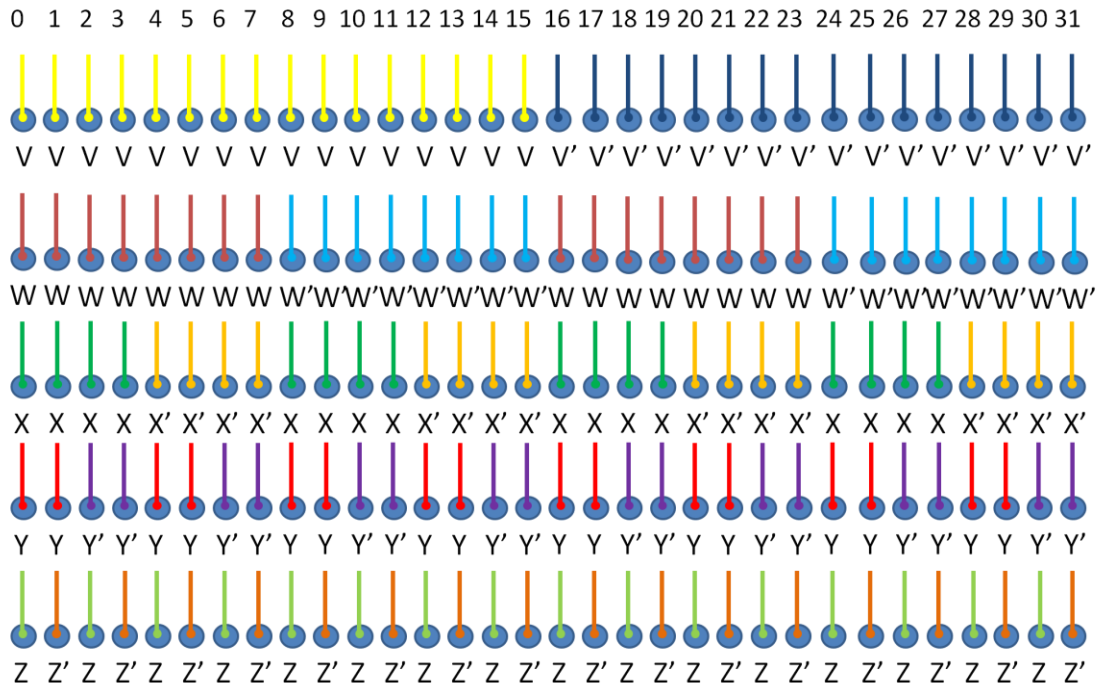


Figure 4.5: Illustration of solution space to be searched during computation for a 5-variable SAT Problem. Each variable is immobilized via applying a constant current to the selected pads. After 10 activations the solution space is obtained.

After 10 immobilizations, the solution space can be determined. At each immobilization, 16 pads are activated, as seen in Figure 4.5. The 1st 16 pads in the 1st row are activated once, and variable v is immobilized. Then the 2nd 16 pads in the 1st row are activated to immobilize v' sequences. After 10 operations, the entire space has been searched, and the activated sites are recorded.

In the 2nd project, the following 5-variable, 6-clause SAT problem was asked to the solution space with the same logic as the 1st project.

$$(v \vee w \vee y) \wedge (w' \vee x \vee z) \wedge (x' \vee y') \wedge (v' \vee z') \wedge (v \vee w' \vee z') \wedge (w)$$

For each query the corresponding complementary sequences were introduced to the array, and after hybridization the image and the fluorescence intensity of the array were recorded. In Figure 4.6a the array is illustrated when query $(v \vee w \vee y)$ is asked via introducing complementary strands $C(v=1)$, $C(w=1)$, $C(y=1)$ and reporter sequences. In Figure 4.6b the image of the array after hybridization reveals that columns 0, 1, 4 and 5 fail to satisfy this query as indicated with a red circle.

In the Figure 4.7, it can be seen easily which columns cannot be the answer of the second SAT problem. When putting the pictures one after the other, the answer of the SAT problem is pads 12 and 28, indicating $(v=0, w=1, x=1, y=0, z=0)$ and $(v=1, w=1, x=1, y=0, z=0)$.

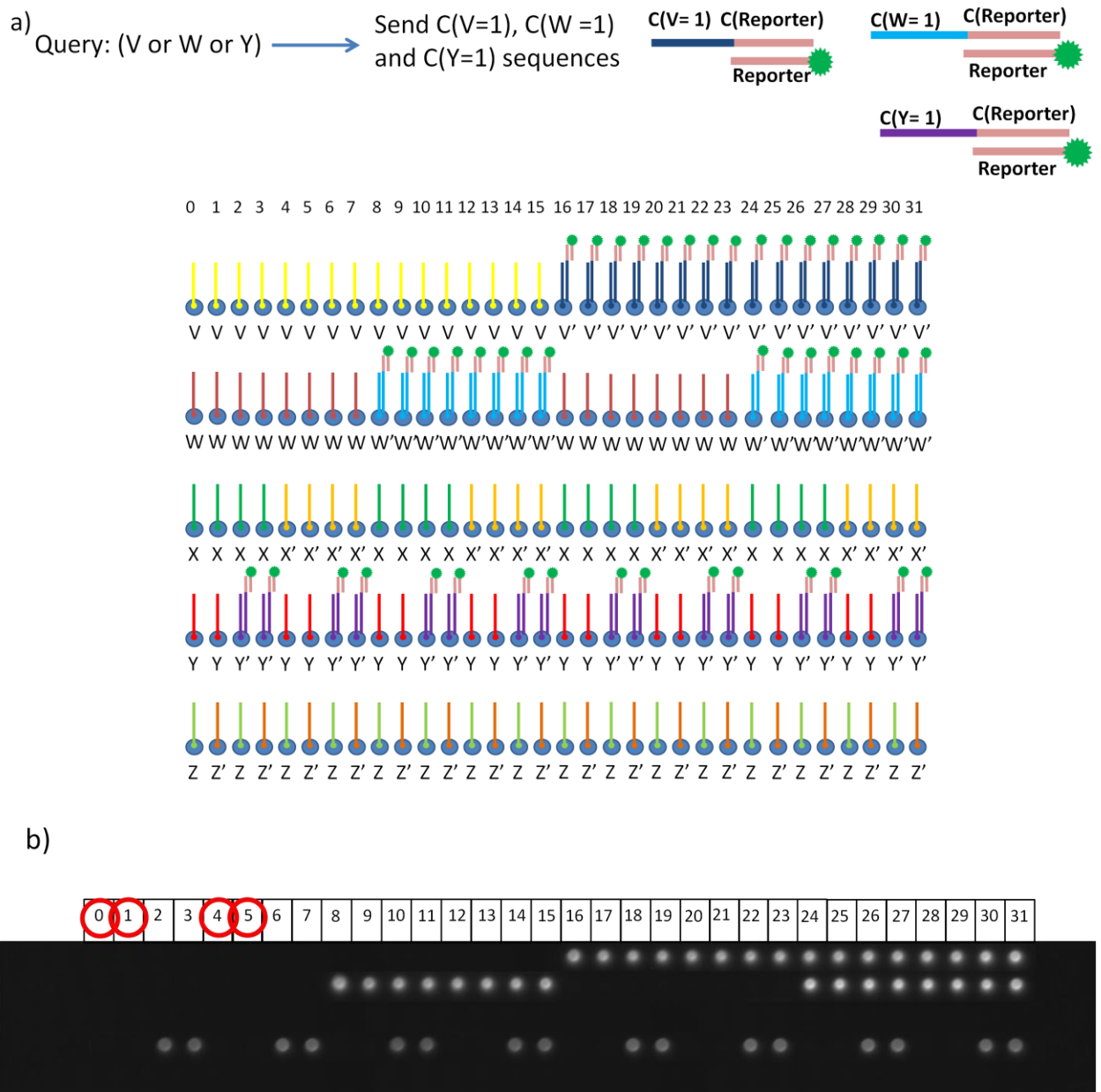


Figure 4.6: Illustration and experimental result of an example query. a) Illustration of asking clause $(v \vee w \vee y)$ to the solution space. b) After correct hybridization, columns 0, 1, 4 and 5 do not have any pads showing fluorescence signal indicating they cannot satisfy the query.

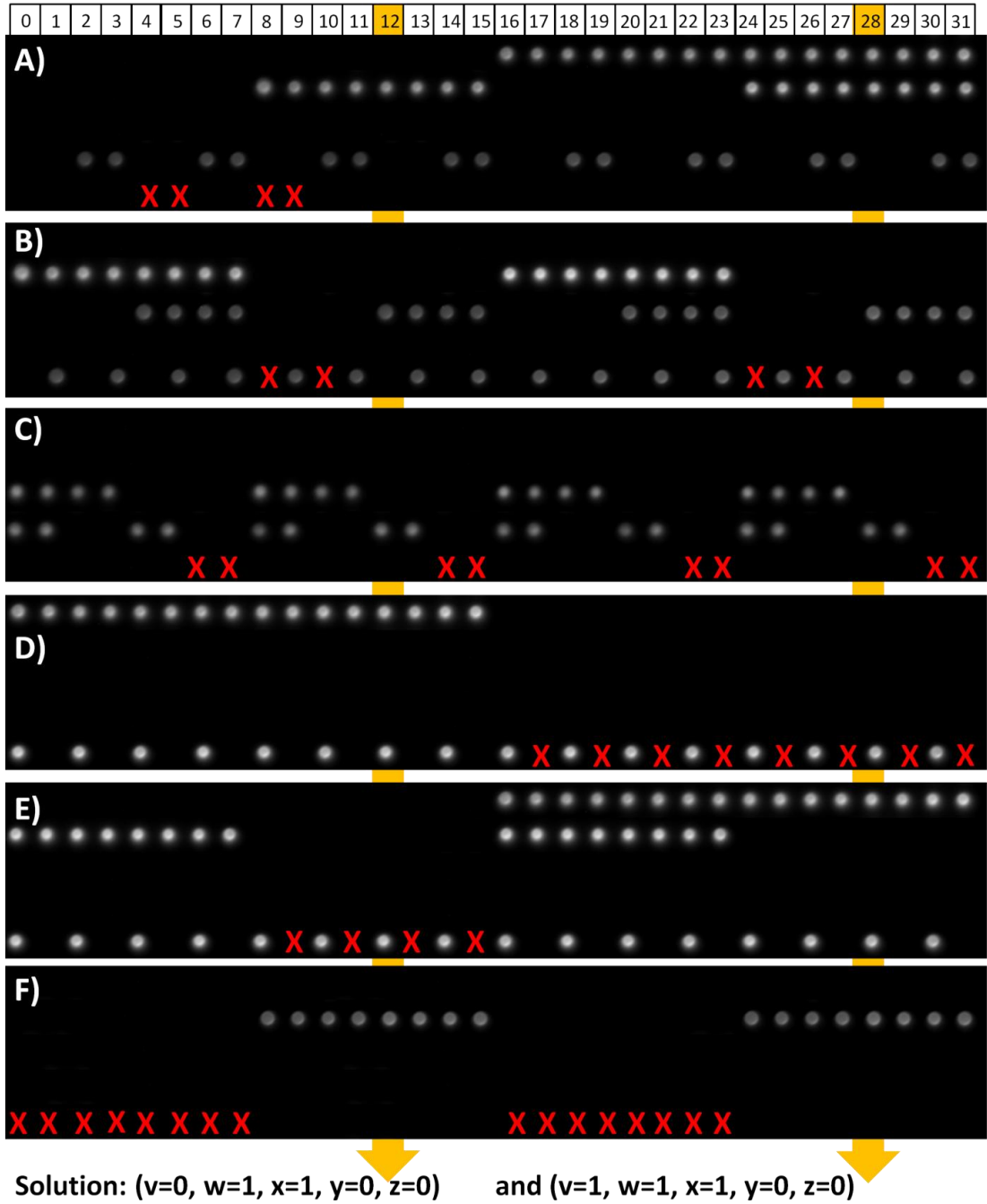


Figure 4.7: Each clause is a query, introduced to the solution space and image is recorded. At A) $(v \vee w \vee y)$, B) $(w' \vee x \vee z)$, C) $(x' \vee y')$, D) $(v' \vee z')$, E) $(v \vee w' \vee z')$ and F) (w) clause is introduced. After temperature-controlled washing of the array and eliminating the mis-hybridized pads, the answer of the corresponding clause can be seen at each image. When the images of each query are brought together, the answer of the second SAT problem is obtained. In this case the answer is pads 12 and 28 satisfying all 6 clauses.

In this project, it became clear that keeping track of the pads becomes more difficult for the user as the size of the problem increases. Therefore, in addition to recording the signal levels from each pad, the columns are marked. The columns are marked with “1”, if there is 1 or more fluorescing pad and with “0” if there are no fluorescing pads. In Figure 4.8 the result of this marking is seen. The correct answer of the SAT problem is obtained much more easily than in Figure 4.7 (Because each column is a possible solution of the problem, this record-keeping helps). Sequences 12 and 28 only have 6 bars, and all the remaining sequences have 5 or fewer bars, indicating they cannot be answer of this SAT problem.

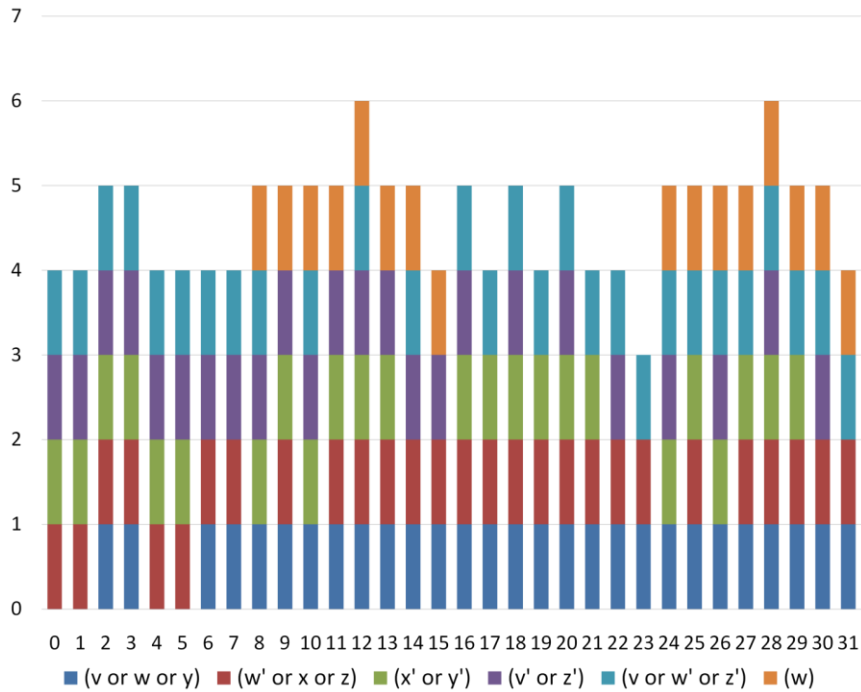


Figure 4.8: After each query the columns with 1 or more fluorescing pads are marked with “1” and columns having no fluorescing pads marked with “0”. When putting the marks on top of each other indicating one query after the other, the histogram shows which sequence satisfies which query. This histogram clearly shows that only sequences 12 and 28 satisfy all 6 clauses.

Discussion

One of the fundamental problems in applications with DNA is the potential for nonspecific binding – i.e., the equivalent of a “false positive” signal. One goal in this study is introducing CMOS technology enabled surfaces, where electric field at the desired pad can be activated to enable electrophoretic control of the movement of DNA molecules. This active control of DNA transport in the desired direction reduces the immobilization time of oligos at the activated locations from several hours to 1 minute and promotes fast hybridization reactions. Here this accelerated specific binding lead to reliable and controllable computation with DNA, as the 2 SAT problems demonstrated.

This approach has some significant advantages over previous experimental techniques, including lower costs, shorter operating time, reusable surface, and simple experimental steps. Compared to the seminal surface based DNA computing paper, where Liu et al. used 2^n unique DNA strands in their algorithm, this study employed $4n$ unique oligos (n represents the number of variables in the 1st project) because DNA strands are attached in tandem. In Liu et al.’s algorithm during “destroy” operation, *E. coli* exonuclease is employed to eliminate surface-bound single strand DNAs. This introduces false signals due to enzymatic reaction efficiency, and it adds cost. Instead of “mark”, “destroy” and “unmark” steps, only mark and read steps are used in the CMOS computation method reported here. The result of the computation is recorded via taking the image of the array. Unmarking the solution space for the next computation enables employing the same surface for the next computation. Because the immobilized oligos can be used for the

next computation – and during computation no enzyme is employed – the cost is reduced and the solution space is regenerated for the next computation with a simple washing operation. Moreover, because results are read through a simple optical method (the CCD camera), the need for additional PCR amplification is eliminated. This step in other studies introduced an additional source of error to the computation due to false-positive signals and non-uniform amplification. During this study it has been realized that the array can be exposed to query solutions several times (array was exposed to the solution 20 times here), and this gives the researcher the opportunity to interrogate the immobilized oligos several times with different oligos. During DNA computation the array surface can be employed without loss of the signal due to the strong biotin-streptavidin bond, which immobilizes the DNA strands to the pads. Loss of signal was an important problem in other studies.^{7,8}

In the 2nd project, one variable is immobilized on a pad and more pads are employed, this can give advantage of reducing the immobilization time, especially for future applications. After 10 activations, sequences for 5 variable SAT problem are immobilized (One activation per variable and at each activation oligos are immobilized 16 pads). Even though the case studies employed here used small numbers of variables, it is open to scalability. If a 20-variable problem is considered as an example, such as the one Braich et. al solved, the method in the 2nd project requires 40 activations, which takes approximately 60 minutes. The need of more than 1,000,000 pads can be reduced with a smarter algorithm, but even this number can be obtained with simple lithography because all these pads only need 40 different underlying control contact. Such a chip can be

fabricated today on a substrate of approximately 2 cm x 2 cm. This chip would be just a scaled-up version of the current platform, and at its current stage it can solve the current world record DNA computing problem in the same day rather than after weeks.

Conclusion

By using an advanced biochip technique, a new DNA Computing model is presented to solve 2 simple SAT problems. Integration of a CMOS platform and DNA for a completely non-biological purpose is demonstrated. This hybrid system integrating silicon processing and biological tools has the potential to deliver sufficient control to enable a feasible approach to rapid computing.

The microchip directs the transport, concentration, and attachment of DNA strands to selected pads to create an array of DNA samples. Here it was the solution space to be searched for the SAT problems. Through control of the electric field, the microchip enabled accurate identification of the correct solutions to 2 SAT problems using fluorescently labeled DNA reporter probes.

This process eliminates the need for PCR and enzymes, and thus error rate and cost are decreased. The research also demonstrated the reliability and stability of the CMOS surface. Automated systems gave researchers some significant advantages in cost and operating time (from days to hours), and the reusable surface and simple experimental steps gave the result of SAT problem after a couple of hours.

Moreover, even though the system is tested with 2 SAT problems here, this style of problem solving can be applied to other DNA computation problems such as memory and 0-1 integer programming (as in 0-1 knapsack problem and DNA2DNA applications). These are typical NP-hard problems with applications to real-life computational challenges such as financial management and business planning. Moreover, DNA2DNA computations which involve the use of DNA computers to perform operations on unknown pieces of DNA without having to sequence them first have an incredible application potential.²²⁻²⁷

Other than DNA computing, several fields that rely heavily on the hybridization of sets of short oligos for both biological and nonbiological applications can utilize this tool. These include the hybridization adsorption onto DNA arrays for biosensor applications, the creation of biomolecular-based computational systems, and the formation of novel nanostructured materials with unique optical and transport properties.²³⁻²⁷ The highly predictable hybridization chemistry of DNA, the ability to completely control the length and content of oligos, and the wealth of enzymes available for modification of DNA make the nucleic acids attractive for all of these applications.²⁴⁻²⁹ The significance of this research, as Adleman pointed out, is that molecular computers will have direct value and effect in biology, chemistry and medicine more than in computer science.⁸

DNA computing has expanded our horizons of what computation is. Surface-based computing emerged as a promising approach in DNA Computing, but in order to carry DNA Computing forward to new venues, improved surface attachment chemistries and

better control of the chemical as well as biological processes is required. One new tool is introduced here to this research field.

Experimental Details

Structure, Fabrication and Working Mechanism of the Microarray

Nexogen, Inc. has further developed on top of Nanogen's electronic microarray technology that utilizes electric fields to accelerate and manipulate biomolecules such as DNA, RNA, and proteins on a microarray surface. Each test site on the microarray has an underlying platinum electrode, which can be activated independently due to the integrated CMOS structure. The integrated CMOS allows precise control over the voltages and currents, which in turn allow accelerated immobilization of biomolecules onto the individual electrode sites. This conventional CMOS structure is fabricated in a commercial foundry using 17 level process steps. Afterwards, the surface is passivated while growing an insulating layer of silicon dioxide, and vias are etched down to the interconnect metal. Using photolithography and deposition processes, 20 nm titanium and 100 nm platinum are deposited and selectively etched to form electrodes, additional electrical wirings and contacts. Next, a silicon oxide insulating layer is deposited by plasma-enhanced chemical vapor deposition, finally producing the individual electrodes at each site on the surface of the chip. Each electrode is 50 μm in diameter, and the center-to-center distance between the two electrodes is 150 μm . On the surface of the array, a 1-2 μm thick hydrogel permeation layer containing streptavidin is spin coated and, in later applications, deposited by microreaction molding.³⁰⁻³⁵ The idea behind using

this layer is, first of all, to protect the DNA strands from the undesirable electrochemical effects at the platinum electrode surface during activation of the pads. Second, the permeation layer assists the attachment of biotinylated oligos through biotin and streptavidin binding. This allows the electronic microarrays to concentrate the oligos at the specific pad 1000 times more than low diffusion dependent passive hybridization arrays.³⁵

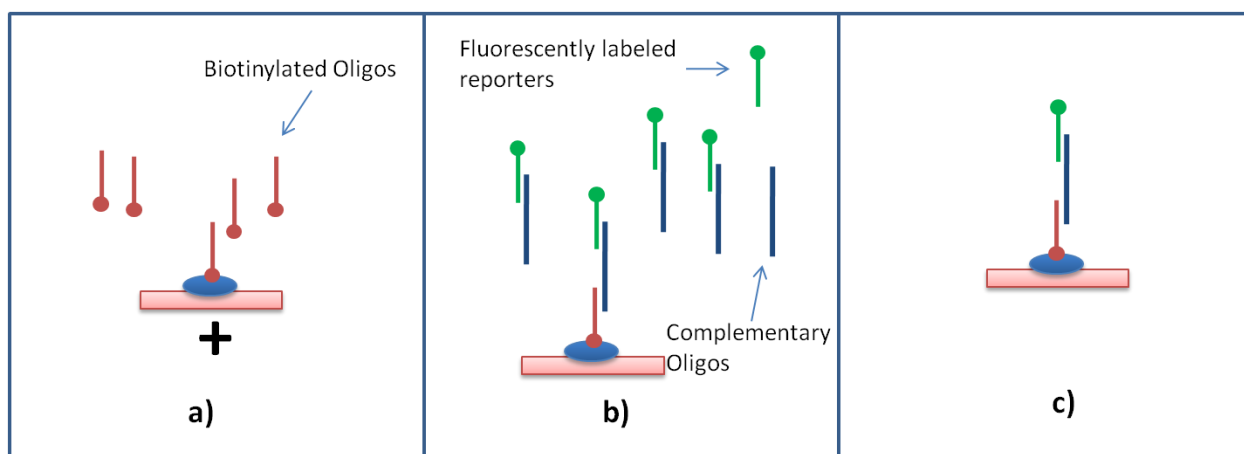


Figure 4.9: a) b) and c) illustrate the working mechanism of the microarray platform with 1 pad, which is using complementary hybridization of matching DNA strands.

In Figure 4.9 the working mechanism of the microarray platform is illustrated. In Figure 4.9a) the microarray is exposed to a solution containing biotinylated oligos. The pad is positively charged, attracting oligos and binding them to the pad. When the charge is removed and the microarray is flushed with buffer, the bound DNAs will remain. In Figure 4.9b), the microarray is exposed to a solution containing complementary oligos and green fluorescently-labeled reporters. Passive hybridization to the immobilized oligos is accomplished. No charge is applied to the pads. In Figure 4.9c) the microarray is heated to an oligo-specific temperature and washed with a high-salt buffer. The unbound

DNAs are washed away and the bound DNA complexes remain. The fluorescent signals are measured from each pad.

Immobilizing DNA Sequences onto the Pads

To immobilize each DNA strand on the platform, 800 nano Ampere constant current on each pad is applied for 60 seconds when introducing 100 nano Molar ssDNA solution to the array. DNAs were diluted in a histidine buffer (114 mM histidine, 142.5 mM 1-Thioglycerol). Due to their negative charged backbone, strands are attracted to the positive charge on the pad. The unattached DNA is removed from the array by washing with histidine buffer. This method is employed for each ssDNA, and 16 different strands are immobilized one at a time onto designated pads and the solution space to search for during computation is obtained.

Design of the Sequences

During the selection of variables, Hamming distance, secondary structures and hairpins are considered. Each variable designed has a T_m between 40C and 45C. The real challenge is keeping the secondary structure at low T_m after putting the variables in tandem. In a regular DNA design the secondary structure in such a configuration is too strong to overcome. Therefore, spacer sequences are added between the variables to minimize the secondary structures and hold the T_m of the solution sequences below 55C.

Washing Protocol used during Hybridization

The following temperature-controlled washing protocol is pursued. The temperature of the array is raised to 60C and hold for 1 minute. Next, the temperature is lowered at each 30 seconds 1C until it reaches 30C. At this point, the microarray is washed with a high-salt buffer (50mM sodium phosphate and 500mM sodium chloride pH7.0). This method removes any weak secondary structures in the immobilized strands as well as in the complementary oligos which may keep them from forming Watson/Crick complexes. Moreover, it prevents unwanted DNA/DNA interactions that may occur between partially complementary sequences, such as between two complementary oligos. Then microarray is brought to 24C, and the fluorescence signals are measured and photographed with a CCD camera. This is the detection system to learn the result of each computation step. This temperature-controlled washing and picture taking steps are repeated until a robust distinguishable signal is obtained from the pads. The imaging system has an excitation wavelength of 525 nm and emission wavelength of 553 nm. In addition to the above procedure, thermally-assisted washing steps are very important. The wash temperature, high-salt buffer used during the wash, and its amount are critical to obtain a robust signal from the pads. After all answers have been obtained, the temperature of the high-salt buffer wash is increased to 60C. This high-temperature washing cycle is repeated until all hybridized sequences are eliminated. This operation, basically unmarking the solution space, cleans the entire array and prepares the array for the next query. These steps are repeated for each clause and these simple annealing and melting steps are the foundations of our computation.

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5. Experimental Demonstration of Hopfield Neural Network using DNA molecules

Abstract: DNA Computing is a rapidly developing interdisciplinary area, but more experimental results will elucidate problems that remain with the current biological tools. In this study, microelectronics and molecular biology techniques are integrated for showing the feasibility of Hopfield Neural Network using DNA molecules. Adleman's seminal paper in 1994 showed that DNA strands using specific molecular reactions can be used to solve the Hamiltonian Path Problem. This accomplishment opened the way for possibilities of massively parallel processing power, remarkable energy efficiency and compact data storage ability with DNA. However, multiple studies have found that small departures from the ideal selectivity of DNA hybridization lead to significant undesired pairings of strands. These in turn lead to difficulties in schemes for implementing large Boolean functions using DNA. Therefore, these error-prone reactions in the Boolean architecture of the first DNA computers will benefit from fault tolerance or error correction methods, and these methods would be essential for large-scale applications. In this study, we demonstrate the operation of six-dimensional Hopfield associative memory storing various memories as an archetype fault-tolerant neural network implemented using DNA molecular reactions. The response of the network suggests that the protocols could be scaled to a network of significantly larger dimensions. In addition, the results are read on a silicon CMOS platform exploiting the semiconductor processing knowledge for fast and accurate hybridization rates.

Introduction

In 1994 Leonard Adleman demonstrated the feasibility of carrying out a computation at the molecular level by using DNA molecules and specific molecular reactions¹. In this pioneering paper, he encoded a small graph in DNA molecules and solved an example of the directed Hamiltonian path problem by using the tools of molecular biology¹.

Adleman's study revealed the possibilities that can be accomplished with DNA in computing. When the ligation or hybridization of two DNA molecules is considered as an operation, approximately 10^{12} operations can be carried out per second with micromoles concentrations. This speed is much greater than the capabilities of today's supercomputers. When the energy efficiency of molecular reactions is considered, 1 J is enough for 2×10^{19} operations with DNA, versus 10^9 operations per J in supercomputers. In the high-density information storage area, 1 gram of DNA can hold about 10^{14} MB of data, which is equivalent to 140 trillion CDs¹⁻³. The fundamental reactions in molecular biology, such as Watson-Crick DNA hybridization, ligation and PCR, are employed as a part of DNA-based computation algorithms in most schemes proposed¹⁻⁵. However, none of these reactions are error free⁵, and DNA computing can benefit tremendously from error-correcting mechanisms. In the search for fault-tolerant or error-correcting methods, neural networks have significant advantages, especially for larger-scale applications in the future^{6, 7}. We applied Mills, Yurke, Platzman (MYP)⁸ network, which is a type of neural network where DNA is used as the substance. This network is effectively the implementation of a Hopfield neural network (HNN)⁹, where the axons and neurons are replaced by DNA molecules. Modeling of such a network is possible using matrix

operations. In the MYP implementation, information consists of sets of oligomers, with each oligomer representing one particular element of information^{8, 10, 11}. Memory matrix elements are then represented by sets of ligated oligomers from the memory vectors. All of this is possible through an extension of Oliver's¹² matrix algebra, which describes a method for calculating the product of matrices using DNA. In this study, we demonstrate the operation of a six-dimensional Hopfield associative memory storing two memories as an archetype fault tolerant neural network implemented using DNA molecular reactions.

Theory

Hopfield Neural Network

Our brains contain about 100 billion units called neurons¹³, where each neuron is connected to thousands of other neurons and communicates with them using electrochemical signals¹³⁻¹⁵. Neurons continuously receive input signals, sum them up in some way, and if the end result is greater than some threshold value, the neuron fires the output signal^{14, 15}. Artificial neural networks (ANNs) are synthetic attempts to simulate this process¹³⁻¹⁶. An ANN consists of a set of computing elements. These elements can be connected in such a way as to store information, perform specific mathematical operations, or find patterns. Information processing capabilities of ANNs and their ability to learn from examples make them efficient problem-solving models¹³⁻¹⁵.

Hopfield neural network (HNN) is suited to memory recall⁹⁻¹¹. Information is stored by presenting data patterns to be stored one by one to the network. The network learns from

examples, and they become memories for the network. Once these memories are stored, it is possible to recall them by presenting a piece of information^{9,13-16}.

In a HNN, the network serves as a content addressable memory⁹. In a standard computer memory such as RAM, memory addresses are supplied and RAM returns the data word stored at that particular address. However, at a content addressable memory also known as associative memory, after data word is supplied, the entire memory is searched whether data is stored anywhere or not. Content addressable memory is a special type of memory and is used in very high speed searching applications. Here this memory application is realized with DNA.

At HNN, the vectors introduced are information, where each vector entry represents a portion of the information to be remembered, such as a pixel in a picture^{8,9}. A particular piece of information (memory or experience in the Hopfield network) is represented by a d-dimensional vector⁹. (1)

$$\vec{V} = \sum_{i=1}^d V_i \hat{e}_i \text{ in a space with basis vectors } e_i (i=1, 2, \dots d). \quad (1)$$

Each component of the memory matrix is constructed from an experience vector⁹. The items of memory are stored in memory by summing the outer product matrices of the memory vectors (2), where each set of vectors $\vec{V}^{(a)}$ (with $a=1, 2, \dots s$) represent a different experience⁹.

$$T_{ij} = \sum_{a=1}^s V_i^{(a)} V_j^{(a)} \quad (2)$$

Information is recalled from the memory matrix by introducing a “clue” vector to the matrix^{9, 13-16}. This clue vector has some of the components missing or set to zero. A particular experience $V_i^{(b)}$, imperfectly represented by a truncated “clue” vector $U_i^{(b)}=V_i^{(b)}$ for $i \leq q$ and $U_i^{(b)}=0$ for $i > q$, is recalled by iterations as below equations 3 and 4 show⁹.

$$X_i^{(1)} = S \left\{ \sum_{j=1}^m T_{ij} U_j^{(b)} \right\} \quad (3)$$

$$X_i^{(2)} = S \left\{ \sum_{j=1}^m T_{ij} X_j^{(1)} \right\} \quad (4)$$

Here the function $S(x)$ is a saturating function such as $\tanh(\lambda x)$ acting separately on each component of its vector argument⁹. It has been proved that if there are enough iterations, it should be possible to recall almost as many memories as there are components in the information vectors, even for a small clue vector. This is due to the pseudo-orthogonality of the information vectors⁹.

DNA representation of HNN

To create fault-tolerant computing algorithms with DNA, Mills, Platzman, Yurke (MYP)⁸ model HNN is researched. This research was undertaken in collaboration with Professor Allen Mills’s group. Axons and neurons are replaced by DNA molecular recognition and matrix operations are performed on the network^{10, 11}. Here, information is represented with sets of DNA sequences. In this system of computation, each vector entry is a set of identical DNA oligomers, which serves as a “basis” for one of the memory vectors⁸. Memory matrix elements are represented by sets of ligated oligomers from the memory

vectors as an extension of Oliver's matrix algebra^{8, 10, 11}, which describes a method for calculating the product of matrices using DNA¹². The connection strengths of the outer products in the memory matrix are formed by construction of a set of ligated vector entries, where each vector entry is ligated to itself in solution¹⁰. The memory matrix, then, is nothing more than that set of all ligated vector entries with themselves, stored in solution and ready to act on an input clue vector. To be able to successively perform Oliver's analog matrix algebra¹² using DNA, we worked with two different vector spaces. These two vector spaces are represented by two independent sets of single-stranded DNA (ssDNA) sequences and their complements, $\{I_i\}$ and $\{O_i\}$, corresponding the basis vectors $\hat{e}_i$ in the input and output vector spaces. The oligomers of the set $\{I_i\}$ are chosen to be minimally hybridizing with the set $\{O_i\}$ to prevent unwanted interactions between the inputs and outputs. Thus there is a pair of determined ssDNA oligos (input and output) assigned to each basis vector. Depending on whether the i^{th} component of the a^{th} vector ($V_i^{(a)}$) is +1 or -1, the $V_i^{(a)}$ will be represented by a specific ssDNA oligo or its complement. So, $\vec{V}^{(a)}$, representing an experience, is nothing but a combination of various determined DNA strands each with the same concentration. In our specific representation we have chosen the $\{I_i\}$ and $\{O_i\}$ as 40 base pair (bp) and 60 bp ssDNA respectively. Each component of the memory matrix constructed from an experience vector $\vec{V}^{(a)}$ is also a DNA strand which we produced by ligating the 5' end of the complement of an input DNA with the 3' end of the complement of an output DNA.

Results and discussion

Two randomly chosen black and white images (Figure 5.1a), each consisting of 6 pixels, are stored in our six-dimensional Hopfield memory, as the experiences. These images are two different six-bit binary information sets. A different set of 40bp (I_i) and 60bp (O_i) DNA strands are employed to represent each white pixel in the input and output vector spaces, respectively. If a pixel is black, the complementary DNA strands corresponding to this pixel are used.

a) Memory 1

Memory 2

b)

$$T_{ij} = \sum_{a=1}^s V_i^{(a)} V_j^{(a)} \begin{pmatrix} 1 \\ -1 \\ 1 \\ -1 \\ 1 \\ -1 \end{pmatrix} (1 \ -1 \ 1 \ -1 \ 1 \ -1) + \begin{pmatrix} 1 \\ -1 \\ -1 \\ 1 \\ -1 \\ 1 \end{pmatrix} (1 \ -1 \ -1 \ 1 \ -1 \ 1) = \begin{pmatrix} 2 & -2 & 0 & 0 & 0 & 0 \\ -2 & 2 & 0 & 0 & 0 & 0 \\ 0 & 0 & 2 & -2 & 2 & -2 \\ 0 & 0 & -2 & 2 & -2 & 2 \\ 0 & 0 & 2 & -2 & 2 & -2 \\ 0 & 0 & -2 & 2 & -2 & 2 \end{pmatrix}$$

Figure 5.1: a) Two randomly chosen black-and-white images and their mathematical representations. These six pixel images are stored in our six-dimensional Hopfield memory as the experiences. b) Mathematical construction of the memory matrix.

For instance, the 1st pixel of both images is represented with I_1 and O_1 , indicating white.

On the other hand, $\bar{I}_2$ and $\bar{O}_2$ are used to represent the 2nd pixel of both images, indicating black. The memory matrix of each six-pixel image is constructed separately. Figure 5.1b shows the mathematic representation of the operation. First, opposite DNA strands of an image in input space are phosphorylated at their 5' ends (Figure 5.2a). Without

phosphorylation, the phosphodiester bond can't be formed during the ligation reaction^{17, 18}. Then, the 5' end of each opposite DNA strand of an image in input space is ligated with the 3' end of each opposite DNA strand of the same image in the output space (Figure 5.2b). These reactions are carried in different vials (Figure 5.3, V1 to V4 for image 1 and V5 to V8 for image 2) to prevent undesired cross-hybridizations. The process results in 100-base-pair-long memory matrix oligos.

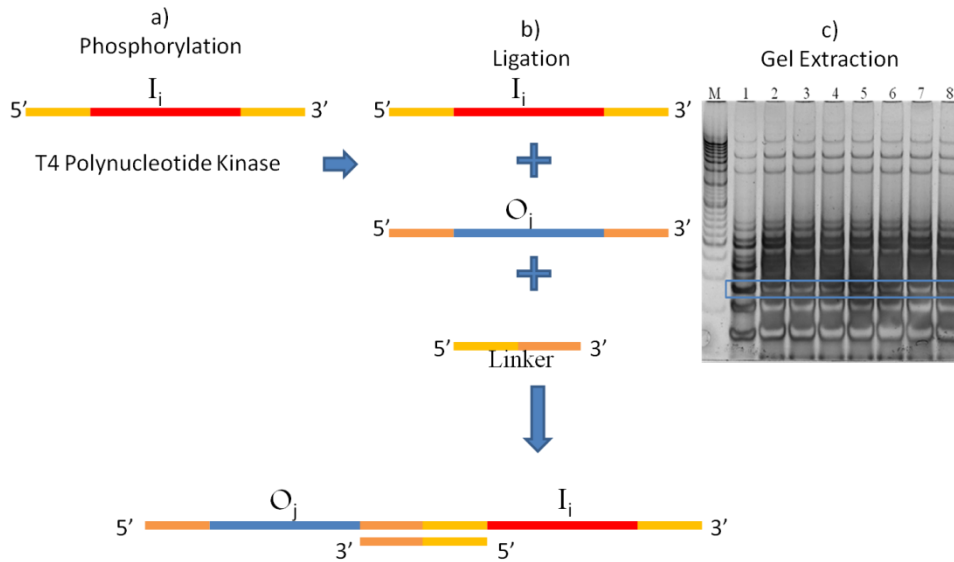


Figure 5.2: Visual description of the experimental procedure to produce memory matrix oligos. a) Phosphorylating 5' ends of input oligos b) Ligating input and corresponding output c) Gel image results obtained at the end of ligation reactions. The bands encircled by blue rectangles represent ligated DNA molecules, the memory matrix oligos.

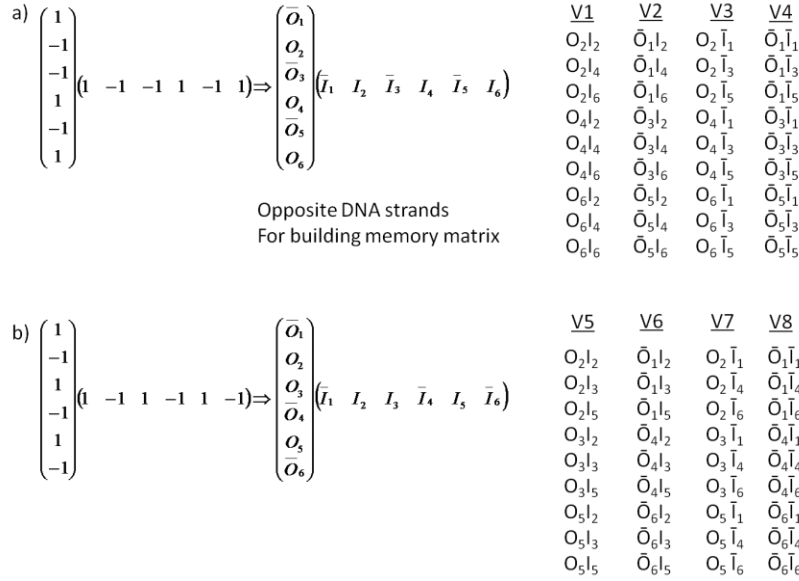


Figure 5.3: A schematic description summarizes the memory matrix production from mathematical form to DNA representation. a) and b) illustrates the conversion of mathematical operations to DNA operations with opposite DNA sequences. To construct the DNA memory matrix, ligation operations are carried in different vials V1 to V4 for image 1 and V5 to V8 for image 2.

There are four different types of memory matrix oligomers (OI's, $\bar{O}I$'s, $O\bar{I}$'s, and $\bar{O}\bar{I}$'s), each of which is generated in different vials, for each image as can be seen in Figure 5.3.

V1 to V8 in Figure 5.3 represents a different vial. Since there are undesired hybridizations, the desired ligated strands are separated from others with a 45-minute 155V gel extraction (Figure 5.2c). After memory matrix oligomers are obtained for both images, the same type of oligomers generated from different images are mixed together namely; V1and V5, V2and V6, V3and V7, V4 and V8.

Now memory matrix is ready and we can make matrix operations on it with the input strands corresponding to a query image. A query image is a truncated version of one of the original memory images, with some unknown pixels (represented here with “?”).

Figure 5.4 shows the mathematical operation performed on the memory matrix. Figure 5.5a shows our query image (?, B, W, B, ?, ?) obtained by truncation of the first memory image (W, B, W, B, W, B). This image is shown in Figure 5.4 as (0, -1, 1, -1, 0, 0) vector. Chemically, our query image is a mixture of DNA strands composed of $\bar{I}_2$, I_3 , and $\bar{I}_4$. The first operation of the query image on memory matrix is to add a definite amount of 40bp input oligomers representing the query image into the memory matrix solutions. If the query input strands are complementary to some of the memory matrix oligomers in a vial, hybridization will take place and the 20bp linker attached to the memory matrix oligomers will denature (Figure 5.5b). Otherwise, there will be no reaction. If hybridized molecules, containing partially double strand DNA (dsDNA), are formed in any of the 4 vials, they will be extended using Klenow Fragment (New England Biolabs, Cat#M0212S, 5 units/ μ l) polymerase in the next step so that 100bp fully dsDNA molecules will be formed (Figure 5.5c). Extending partially dsDNAs are carried in each vial for 1 hour. At the end, 100bp fully dsDNA oligomers are created. After the 100bp fully dsDNA oligomers are formed in different vials, they are separated from other undesired strands by one more gel extraction (Figure 5.5d).

$$X_i^{(1)} = S \left\{ \sum_{j=1}^m T_{ij} U_j^{(b)} \right\} \begin{pmatrix} 2 & -2 & 0 & 0 & 0 & 0 \\ -2 & 2 & 0 & 0 & 0 & 0 \\ 0 & 0 & 2 & -2 & 2 & -2 \\ 0 & 0 & -2 & 2 & -2 & 2 \\ 0 & 0 & 2 & -2 & 2 & -2 \\ 0 & 0 & -2 & 2 & -2 & 2 \end{pmatrix} \begin{pmatrix} 0 \\ -1 \\ 1 \\ -1 \\ 0 \\ 0 \end{pmatrix} = \begin{pmatrix} 2 \\ -2 \\ 4 \\ -4 \\ 4 \\ -4 \end{pmatrix} \Rightarrow Sgn \begin{pmatrix} 2 \\ -2 \\ 4 \\ -4 \\ 4 \\ -4 \end{pmatrix} = \begin{pmatrix} 1 \\ -1 \\ 1 \\ -1 \\ 1 \\ -1 \end{pmatrix}$$

Figure 5.4: Clue vector (0, -1, 1, -1, 0, 0) operates on the memory matrix to recall the original memory (1, -1, 1, -1, 1, -1). Saturation function Sgn operates on the result of the multiplication and brings the recalled memory.

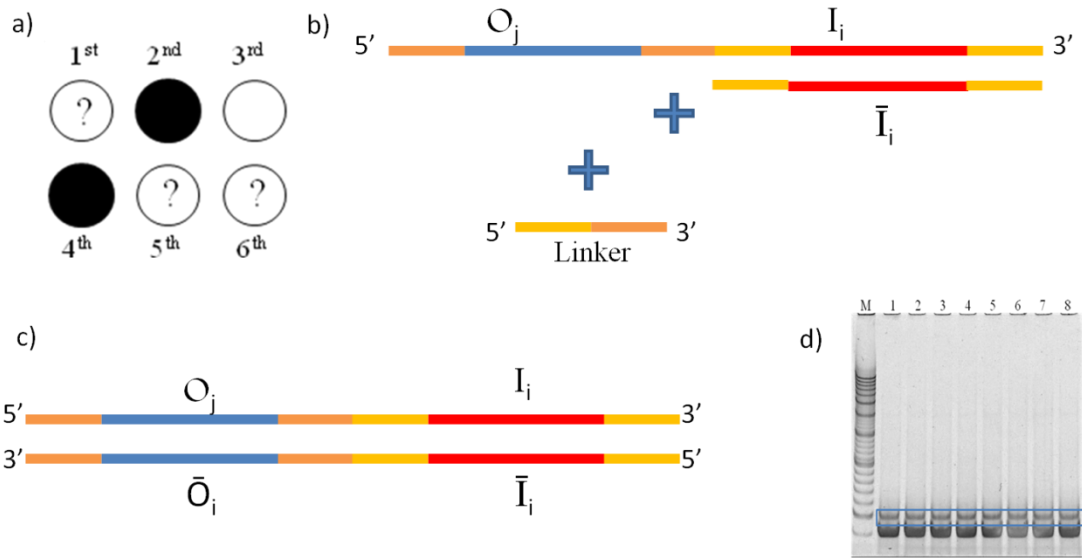


Figure 5.5: a) Query image with 3 unknown pixels is used to recall the 1st image b) Input strands hybridize with memory matrix oligos c) Klenow fragment extends the lower strand and generates a dsDNA d) Gel image results after extension reaction. The bands encircled by blue rectangles represent 100bp dsDNA molecules.

The next step is transferring the extracted solution which includes 100bp dsDNA into a new vial and producing the output strands using ILA (Isothermal Linear Amplification) reaction¹⁹⁻²¹. During this reaction the following steps will occur: Step 1 (Figure 5.6a) Nt.BbvCI (nicking enzyme) cuts the previously extended lower strands of dsDNA oligomers at a point where the extension was started. Step 2 (Figure 5.6b) Klenow Fragment polymerase starts extending the lower strands one more time while it also displaces the previously extended strands which are the output oligomers. Step 3 (Figure 5.6c) more output strands of each kind are produced while repeating steps 1 and 2 continuously (For details of ILA reaction¹⁹⁻²¹). To stop the ILA reaction, the polymerase and nicking enzymes are heat-denatured. Since a query image represented by 3 input strands is used, some output strands and their complements will be produced at the same

time. Those strands hybridize with each other and yield 100bp dsDNA oligomers, which don't cause a problem since they won't be detected during the read out.

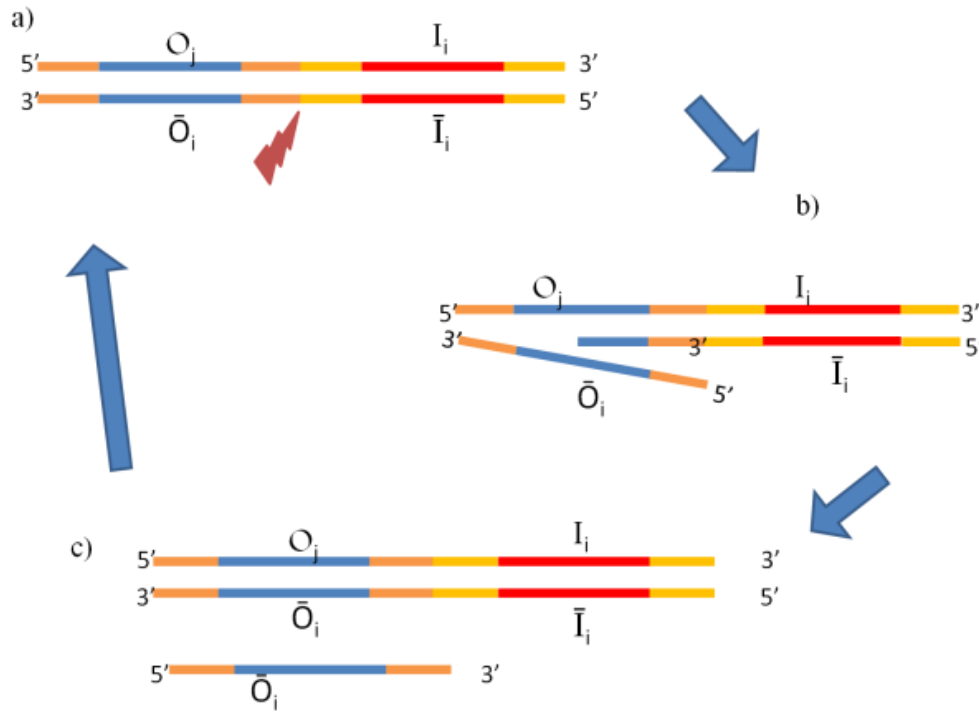


Figure 5.6: Output is produced by ILA reaction a) Nicking (Nt.BbvCI) cleaves the phosphodiester bond b) Klenow Fragment polymerase starts extending the lower strands c) Klenow displaces the previously extended strands.

The read-out process on an electronic microarray²¹⁻²³ can be summarized as follows: First a solution containing TET-primer molecules are added into the final solution produced by ILA reaction. TET-primer can hybridize with output strands because the 20bp at the end of a TET molecule is the complement of 20 nucleotides located at the 3' end of output strands. Second, a solution with a definite amount of Biotin-primer molecules is mixed with the same definite amount of each of $O_1, O_2, O_3, O_4, O_5, O_6, \bar{O}_1, \bar{O}_2, \bar{O}_3, \bar{O}_4, \bar{O}_5$, or $\bar{O}_6$ ssDNA oligomers in different vials. Each type of hybridized DNA strands with biotin primers is immobilized on a different pad of the electronic microarray (Figure 5.7a).

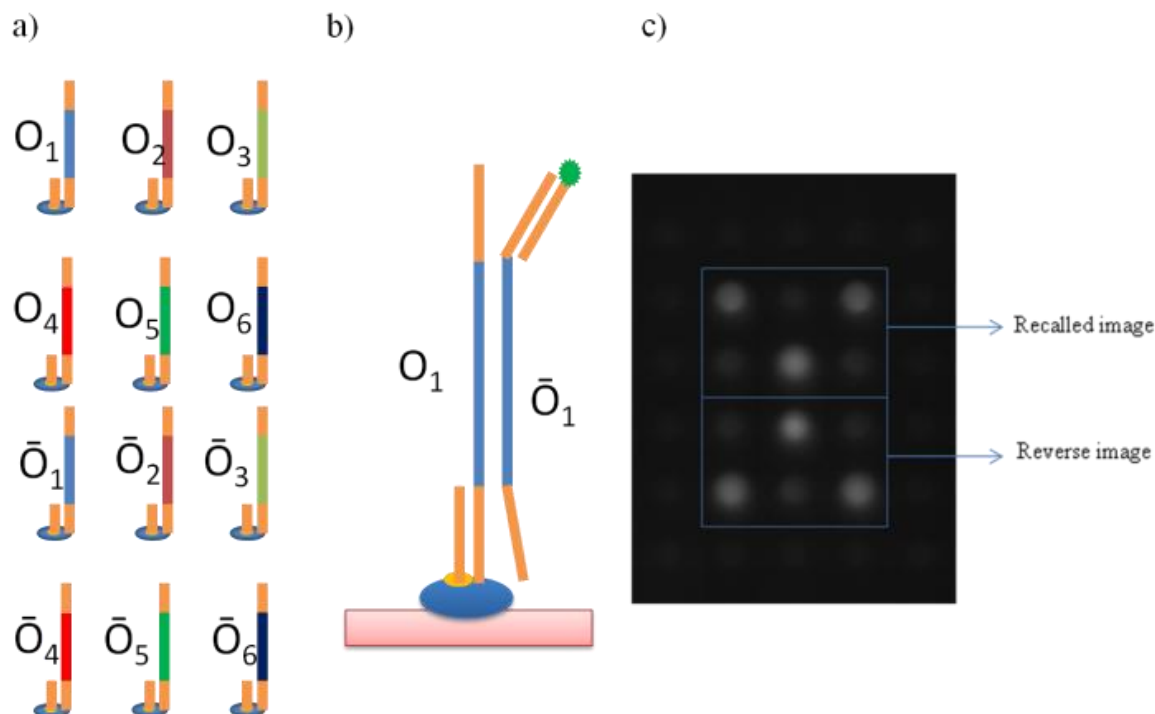


Figure 5.7: a) Illustration of read-out step on electronic microarray. Twelve possible output strands are attached on different pads on electronic microarray. b) Each output strand hybridizes with its complementary. c) Non-complementary and complementary components of the recalled image are obtained in the upper and lower half of 12 pads, proving the successful recalling of the 1st image.

The details of electric field accelerated DNA immobilization can be found in the previous chapters of the dissertation. Finally, the solution prepared in the first step is introduced to the microarray. Each output strand attached to TET-primer will hybridize with its corresponding complement (Figure 5.7b). When excited, TET molecules fluoresce, and we can get the recalled image and its reverse on the electronic microarray (Figure 5.7c). As expected, the first 6-bit image, from which the query image was created, is successfully restored on the microarray as a result of DNA experiments. The task saturation function Sgn performed in the mathematical model, is performed here with the electronic microarray.

Conclusion

We present the experimental implementation of a 6-bit Hopfield neural network using DNA molecules. With a query corresponding to image (?, B, W, B, ?, ?), the first memory image (W, B, W, B, W, B) is successfully recalled. Although the size of the memory images is small, our study indicates the scalability of this kind of implementation because experimental procedures including the memory matrix building operation by ligation reaction is carried out for the first time starting from input and output sequences. In this study, two 6-bit images are used as experiences and a DNA-based memory matrix representing Hopfield associative memory is constructed. A query image derived from the first memory image is employed to operate on the memory to recall the corresponding image. The advantage of DNA-based Hopfield neural network over the theoretical one is the fact that there is no memory lost in DNA-based HNN, whereas such a problem happens in the theoretical model. In DNA implementation, since the memory matrix oligomers are produced and contained in different tubes according to their types, no fully or partially complementary strand are mixed in the same solution. This prevents the loss of memory. To utilize the massive parallelism of DNA and to carry it forward experimental implementation of an associative memory in the form of a Hopfield neural network using DNA molecules is an important step because these applications can become useful in medical diagnostics and disease predictions.

Experimental details

DNA sequences used and their designs

DNA strands used in the experiment are listed below.

I ₁ :	5'- GGA GCA CCT GGC AGG TCT AAC ACC GAC ATT CAG GTG CTC C -3'
$\bar{I}_1$:	5'- GGA GCA CCT GAA TGT CGG TGT TAG ACC TGC CAG GTG CTC C -3'
I ₂ :	5'- GGA GCA CCT GAG GTA CTT GAA CGA TGC GAC CAG GTG CTC C -3'
$\bar{I}_2$:	5'- GGA GCA CCT GGT CGC ATC GTT CAA GTA CCT CAG GTG CTC C -3'
I ₃ :	5'- GGA GCA CCT GTA TGA GAC GCT CTG CTA GAG CAG GTG CTC C -3'
$\bar{I}_3$:	5'- GGA GCA CCT GCT CTA GCA GAG CGT CTC ATA CAG GTG CTC C -3'
I ₄ :	5'- GGA GCA CCT GGG CTC AGA CAA TAC ATC ACG CAG GTG CTC C -3'
$\bar{I}_4$:	5'- GGA GCA CCT GCG TGA TGT ATT GTC TGA GCC CAG GTG CTC C -3'
I ₅ :	5'- GGA GCA CCT GGA CAG TTA GTG CAT ACG GCA CAG GTG CTC C -3'
$\bar{I}_5$:	5'- GGA GCA CCT GTG CCG TAT GCA CTA ACT GTC CAG GTG CTC C -3'
I ₆ :	5'- GGA GCA CCT GAC ATG ACT GCT GTC ATG AGT CAG GTG CTC C -3'
$\bar{I}_6$:	5'- GGA GCA CCT GAC TCA TGA CAG CAG TCA TGT CAG GTG CTC C -3'

O ₁ :	5'- TCA GCA GTT GGT GGC AGG AGA CCG TTG AGT CCG TAT GTC ACT CCT GCC ACC AAC TGC TGA -3'
$\bar{O}_1$:	5'- TCA GCA GTT GGT GGC AGG AGT GAC ATA CGG ACT CAA CGG TCT CCT GCC ACC AAC TGC TGA -3'
O ₂ :	5'- TCA GCA GTT GGT GGC AGG AGG TCA CAT CAC ACG CTT AGG ACT CCT GCC ACC AAC TGC TGA -3'
$\bar{O}_2$:	5'- TCA GCA GTT GGT GGC AGG AGT CCT AAG CGT GTG ATG TGA CCT CCT GCC ACC AAC TGC TGA -3'
O ₃ :	5'- TCA GCA GTT GGT GGC AGG AGC ACG ATC ATC TGG ATA CTG ACT CCT GCC ACC AAC TGC TGA -3'
$\bar{O}_3$:	5'- TCA GCA GTT GGT GGC AGG AGT CAG TAT CCA GAT GAT CGT GCT CCT GCC ACC AAC TGC TGA -3'
O ₄ :	5'- TCA GCA GTT GGT GGC AGG AGC CAC AGA CTC TCA GAT CGT ACT CCT GCC ACC AAC TGC TGA -3'

$\bar{O}_4$:	5'- TCA GCA GTT GGT GGC AGG AGT ACG ATC TGA GAG TCT GTG GCT CCT GCC ACC AAC TGC TGA -3'
O_5 :	5'- TCA GCA GTT GGT GGC AGG AGT TGT GAA CAA TGC TGC CGA CCT CCT GCC ACC AAC TGC TGA -3'
$\bar{O}_5$:	5'- TCA GCA GTT GGT GGC AGG AGG TCG GCA GCA TTG TTC ACA ACT CCT GCC ACC AAC TGC TGA -3'
O_6 :	5'- TCA GCA GTT GGT GGC AGG AGC TAT GAG TCA TAG CGA TGA GCT CCT GCC ACC AAC TGC TGA -3'
$\bar{O}_6$:	5'- TCA GCA GTT GGT GGC AGG AGC TCA TCG CTA TGA CTC ATA GCT CCT GCC ACC AAC TGC TGA -3'
Linker:	5'- CAG GTG CTC CTCA GCA GTT GG -3'
Biotin-primer	5'-/Biosg/ TCA GCA GTT GGT GGC AGG AG -3'
TET-Primer	5'-/TET/TCA GCA GTT GGT GGC AGG AG -3'

Table 5.1: Input (40bp) and Output (60bp) DNA strands with their corresponding complementary strands are shown. The linker is used during the ligation reaction. Biotin-primer and TET-primer are employed for read-out step with the electronic microarray. Each input and output strands correspond to a basis vector in the Hopfield neural network.

At the 5' and 3' ends of both input and complementary input strands GGA GCA CCT G and CAG GTG CTC C sequences are inserted respectively, so ligating input and output strands with the same 20bp linker is possible. In addition, first and last two nucleotides (GG and CC) of invariant 10-mers at the 5' and 3' ends are a partial recognition site of a non-palindromic restriction enzyme (Nt.BbvCI from New England Biolabs, Cat#R0632S, 10 units/ μ l), which performs a nicking operation during the Isothermal Linear Amplification (ILA)¹⁹⁻²¹ reaction. The other 20 nucleotides placed in the middle section of 40 bp input strands are different for each oligo.

Each 60bp output strand and its complementary sequence has TCA GCA GTT GGT GGC AGG AG and CTC CTG CCA CCA ACT GCT GA at the 5' and 3' end, respectively. The remaining 20 nucleotides in the middle of each strand are different.

During the design of input strands, 10 identical base pairs (bps) are placed to the end of the sequences for ligation reaction. But for the output strands 20 bps of identical sequences are placed at the ends of each output strand so that the same biotin-primer and TET-primer ssDNA can hybridize with all output strands. Biotin-primer and TET-primer are necessary to get the final read out on the electronic microarray. The DNA sequences designed with these requirements are purchased from Integrated DNA Technologies (IDT). All strands are diluted with Nuclease-free water (DEPC-Free) after purchase.

Memory matrix production

The memory matrix of each 6-pixel image has to be constructed separately starting with the opposite sets of strands. For instance, the opposite sets of strands for the 1st image are ($\bar{I}_1, I_2, \bar{I}_3, I_4, \bar{I}_5, I_6$) and ($\bar{O}_1, O_2, \bar{O}_3, O_4, \bar{O}_5, O_6$) in the input and output spaces, respectively. First, opposite DNA strands of an image in input space are phosphorylated at their 5' ends by T4 Polynucleotide Kinase^{17, 18} (New England Biolabs, Cat#M0201S, 10units/ μ l). Phosphorylation reactions are carried out in a reaction mixture with a total volume of 50 μ l containing 50 mM Tris-HCl (pH 7.5 at 25C), 10 mM MgCl₂, 1mM ATP, 10 mM Dithiothreitol, 15 units T4 Polynucleotide Kinase, 3 μ M each input strand, and Nuclease-free water. After vortexing, mixtures are incubated at 37C for 30 minutes, then, heated at 65C for 20 minutes to deactivate kinase. Next, the 5' end of each opposite DNA strand of an image in input space is ligated with the 3' end of each opposite DNA strand of the same image in the output space. Ligation reactions are carried out in a reaction mixture with a total volume of 50 μ l containing 50 mM Tris-HCl (pH 7.5 at 25C), 10 mM

MgCl₂, 1mM ATP, 10 mM Dithiothreitol, 600 units T4 DNA Ligase (New England Biolabs, Cat#M0202S, 400 cohesive end units/μl), 1.2 μM each input and output strands, 3.6 μM 20bp linker, and Nuclease-free water. After vortexing, mixtures are incubated at room temperature for 2 hours. Note that each memory matrix oligomer is a 100mer attached to 20bp linker as a partial lower strand. Since there are undesired hybridizations, gel extraction is needed to separate desired ligated strands from others. After the memory matrix oligomers are obtained for both images, the same type of oligomers generated from different images are mixed together.

Operating on the memory matrix

Operating on the memory matrix by using the input strands ($\bar{I}_2$, I_3 , and $\bar{I}_4$) that corresponds to a query image (?, B, W, B, ?, ?) is outlined below.

The first operation of the query image on memory matrix is to add a definite amount of 40bp input oligomers representing the query image into a sample of the memory matrix solutions stored in the 4 different vials with 10xNeBuffer 2 (New England Biolabs). If the query input strands are complementary to some of the memory matrix oligomers in a vial, Watson Crick hybridization will take place and the 20bp linker attached to the memory matrix oligomers will denature. Otherwise, there will be no reaction. Hybridization reactions are carried out in a reaction mixture with a total volume of 80 μl containing 10 mM Tris-HCl (pH 7.9 at 25C), 50 mM NaCl, 10 mM MgCl₂, 1 mM Dithiothreitol, 0.5 μM $\bar{I}_2$, 0.25 μM I_3 and $\bar{I}_4$, one of 4 types of memory matrix strands, and Nuclease-free water. After vortexing, mixtures are incubated at 55C for 1 hour. If hybridized molecules,

containing partially dsDNA, are formed in any of the 4 vials, they will be extended using Klenow Fragment (New England Biolabs, Cat#M0212S, 5 units/ μ l) polymerase, so that 100bp fully dsDNA molecules will be formed. The extension reaction is carried out in a reaction mixture of 5 units Klenow fragment polymerase, 3 μ l Deoxynucleotide solution mix (dNTP) (New England Biolabs, Cat#N0447S, 10 mM), and 0.44 μ l 10xNeBuffer. After vortexing, mixtures are incubated at 37C for 1 hour. At the end of this process, 100bp fully dsDNA oligomers are created.

After the 100bp fully dsDNA oligomers are formed in different vials, they are separated from other undesired strands with one more gel extraction. The electrophoresis runs in a 8% polyacrylamide gel for 65 minutes at 155 V.

Next, the extracted solution including 100bp dsDNA template is transferred into a single new vial for ILA reaction¹⁹⁻²¹. ILA reaction is used to increase the amount of the output strands for detection via microarray. During this reaction the following steps will occur: (1) Nt.BbvCI (nicking enzyme) cuts the previously extended lower strands of dsDNA oligomers at a point where the extension was started. (2) Klenow Fragment polymerase starts extending the lower strands one more time while it also displaces the previously extended strands which are the output oligomers. (3) Steps 1 and 2 are continuously being repeated so that more output strands of each kind are produced. T4 Gene 32 single-strand binding protein (New England Biolabs, Cat#M0300S, 10 μ g/ μ l) is added into the ILA reaction mix to ensure that undesired fragment strands causing difficulty in further steps are not produced¹⁹⁻²¹. The ILA reaction is performed in 50 μ l mixture containing

10 mM Tris-HCl (pH 7.9 at 25C), 50 mM NaCl, 10 mM MgCl₂, 1 mM Dithiothreitol, 25 units Nt.BbvCI nicking enzyme, 5 units Klenow Fragment polymerase, 10 µg T4 Gene 32 ssBP, 1000 µM dNTPs, 15 µl solution of 100bp dsDNA and Nuclease-free water. After vortexing, mixtures are incubated at 37C for 1 hour. In order to stop the ILA reaction, we heat-denature the polymerase, ssBP, and nicking enzyme 80C for 20 minutes and cool down to room temperature. At the end of this reaction, output strands and their complementary strands are produced corresponding to the image we wanted to recall.

The read-out process

Each output strand (O₁, O₂, O₃, O₄, O₅, O₆, $\bar{O}_1$, $\bar{O}_2$, $\bar{O}_3$, $\bar{O}_4$, $\bar{O}_5$, and $\bar{O}_6$) is hybridized with Biotin-primer in a different vial in a solution of 100 mM Histidine and 120 mM 1-Thioglycerol. Each vial contains 1µM biotin-primer and 0.5 µM one of 12 output DNAs. Each type of output strand is immobilized on a different pad on the microarray²¹⁻²³ while applying 800 nA constant current for 60 seconds. Afterwards, the array is washed with histidine buffer and water twice to remove the non-attached DNA strands.

Next, output strands obtained by ILA reaction are hybridized with TET-Primer molecules mixed in a single vial are introduced to the array. 100 µl reaction mix contains 30 mM sodium phosphate, 300 mM NaCl, 1 µM TET-primer, and 69 µl solution of net output strands with indefinite concentration. Solution is dispatched on the array two times each in 50 µl to increase the amount introduced to the array. After introducing the solution to the array, temperature is increased to 60C and held constant for 3 minutes. Afterwards, temperature is decreased every minute 1 °C until 30C is reached. At 30C the array is

washed with 50 μ l high-salt buffer (50 mM sodium phosphate and 500 mM NaCl).

Finally, the temperature is decreased to 24C for imaging. TET molecules are excited with a 525nm green light. Fluorescent signals coming from pads are measured and the microarray image is taken.

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6. Conclusion

DNA nanotechnology is the field of nanotechnology that utilizes the unique structure and properties of DNA, where DNA is not employed as biological material carrying genetic information but used as an engineering material. DNA nanotechnology has various applications but mostly used in DNA computing and molecular self-assembly. In this dissertation these two areas are investigated. I truly believe that DNA nanotechnology is one of the wonderfully fruitful areas in the field of nanotechnology. I presented in this dissertation DNA functionalization of CNTs and their electrical properties, fabrication of NWs, their DNA functionalization for Lock and Key LithographyTM and manipulation of NWs inside solution with an electronic microarray. I had the opportunity to research DNA computing during my graduate studies. DNA computing is a fast developing interdisciplinary area and uses generally biomolecular computing. In this dissertation microelectronics and molecular biology techniques have been integrated to introduce a new DNA Computing platform. An electronic microarray platform is adopted to perform DNA computing. The information present in an image is encoded through the concentrations of various DNA strands via selective hybridization and decoded on CMOS platform to recreate the original image. 2 Satisfiability (SAT) problems were solved via 2 different methods. The approach, I proposed here eliminated the need for PCR (Polymerase Chain Reaction) and enzymes, and as a result error rates and cost are decreased. Overall, this technique shows some significant advantages over previous experimental techniques such as short operating time, reusable surface and simple experimental steps. Finally, microelectronics and molecular biology techniques are

integrated for showing the feasibility of Hopfield Neural Network using DNA molecules. Six dimensional Hopfield associative memory storing various memories is demonstrated as an archetype neural network using DNA molecular reactions. The results are read on electronic microarray platform which opens the semiconductor processing knowledge for fast and accurate hybridization rates. The research under the umbrella of this dissertation is expected to have broad implications for next-generation functional materials as nanoscale building blocks and the proposed surface based DNA computation approach can bring the hybrid concept of silicon compatible DNA computing to realization. Integration of a CMOS platform and DNA for a completely non-biological purpose has the potential to greatly affect the future applications.