NUCLEIC ACID ELECTRONICS
: FROM CHEMISTRY TO CIRCUITS

By

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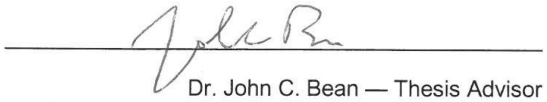

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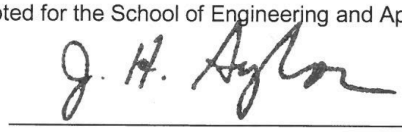

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CHAPTER I.

Introduction

For the past decade, constant warnings of the end of Moore's Law have egged on the development of nanoelectronics. Today, nanoelectronics has developed a range of nano-scale, working, transistor-like devices in the laboratory. But these demonstrations show single functional devices or a mere handful of devices. Microelectronics achieved the same amount of complexity more than a half a century ago. And nanoelectronics must make a quantum leap forward in complexity to merit equal consideration. This will not be possible with the range of tools nanoscience currently depends on. Researchers must shift their focus to techniques that can order nano-objects in complex ways, over a long range. The purpose of this thesis is to look into bio-inspired alternatives that *can* do this, specifically those enabled by biological self-assembly. We focus on how to produce the levels of complexity needed for successor technologies, which is possible via biological self-assembly and biochemistry. This proposal is an intentional and radical departure from molecular electronics and from the majority of nanoelectronics work to date. Our "out of the box" approach seeks to bridge the gap between biology and electronics at the single-molecule level.

A. Defining the box: a short history of electronics

The conventional approach for advancing microelectronics is to (1) add complexity to the circuit, (2) miniaturize devices, and (3) increase the speed. CMOS structures were the first to allow the incremental addition of complexity on integrated circuits after vacuum tubes and bipolar junction transistors. As CMOS-based fabrication improved with optical technology, photolithography facilitated the development of scaling techniques from the 1980s to around the year 2000. With photolithography, the key to making *faster* transistors meant building *smaller* transistors, as velocity and size are related. Shortening the wavelength of patterning light and using other fabrication techniques made smaller devices. Because photolithography is diffraction-limited, 436-nm light formed ~500-nm-channel transistors, and 365-nm light made transistors with ~400-nm features, and so on. Over time, photolithography grew an abundant toolkit: and by "toolkit" I mean the microcosm of laboratory techniques that can be used to manipulate and tailor parts of the process. As the roots of certain scaling techniques took hold, Moore's prediction became an expectation and a driver for the integrated circuit (IC) industry.

The last decade has witnessed how physics has helped push sub-micron technology to its limits. According to Intel, the IC industry used roughly the same scaling techniques until around the year 2000. But, practically every assumption governing the device physics of long-channel transistors eventually broke down. As each assumption grew irrelevant, the new short-channel transistors operated more and more differently. Changes in applied voltages and other staple values became a requirement to insure robust operation. But power dissipation problems now threaten the robust operation of short-channel transistors. This is relevant because in device evolution, power dissipation limitations are responsible for the displacement of previous technologies. And CMOS technology is approaching its power consumption limit fast: its power density dissipated is approaching the heat of a rocket nozzle [1]!

Today, the extension of photolithography depends on an increasingly sparse toolkit of tricks and techniques. To penetrate beyond the sub-micron level, Intel depends on photolithography tricks like immersion lithography and double patterning [2]. Besides photolithography, the breakdown of device physics requires manufacturers to now include other incremental changes in materials, such as hafnium oxides to prevent quantum tunneling through atomically-thin layers. CMOS can only survive as long as chipmakers can adjust their current toolset to meet the next node of Moore's Law. Because of the breakdown of device physics, IC fabrication companies are even considering architectural changes. To battle all the repercussions of short-channel effects (SCE), companies have to choose between Fin-FETs and ultra-thin body (UTB) transistors on silicon-insulator (SOI). By going 3-dimensional, the Fin-FET architecture overcomes SCEs by physically surrounding the channel. On the contrary, the UTB on SOI transistor depends on an ultra-thin body buried in insulator to combat SCEs [3]. Such feats in engineering buy silicon-based technologies some time, but not much. The physical limits of silicon-based atomic structures could be reached by 2020; so nevertheless, this is a good indicator that change is upon us.

In the worst-case scenario, an upper limit caps the future growth of photolithography and the associated toolkits, as we know it [4]. So what will happen after that? How will the IC fabrication industry maintain robustness and the reliability of these devices? Despite the recent fundamental changes in device physics and architecture, power dissipation issues will still plague next-generation silicon-based technologies. To truly escape the limitations of silicon-based solutions, using radically new concepts should be given serious consideration. A reliable replacement technology not based on silicon has yet to arrive because researchers rarely consider complexity.

The ability to add complexity is an often-ignored litmus test for technology development viability. Yet, academic research continually investigates and regularly uses other less-scalable lithographic techniques, e.g. immersion lithography, electron beam lithography (EBL), AFM lithography, STM lithography, etc. But some of these lithography techniques cannot be used to add complexity because these technologies compromise throughput (and the processing time for a more complex technology) for resolution. In other words, higher resolution systems have limited throughput because they work serially. These processes are markedly less automated than photolithography, as they must manipulate point after point. In contrast, the exposure process of photolithography projects a mask's pattern simultaneously over the whole chip. In order to truly replace CMOS technology in its full glory, high resolution and high throughput are both necessary, from a technology development standpoint. And to come up with a technique that exhibits both high resolution and throughput, we really must shy away from using these technologies to create what we need. And because these technologies defeat the purpose of trying to add complexity, throughout this paper, we will generally refer to these technologies as "anti-complexity".

This thesis means to inspire a new genre of alternative electronics, specifically, nucleic acid-based molecular electronics. Nucleic acid electronics has an edge because this field can use recent developments in DNA-related biochemistry to its advantage. So considering what constitutes the box of modern-day electrical engineering, the purpose of this thesis is to think outside of the box.

B. Complexity separates nanoscience from nanotechnology

Researchers have done a lot of great nanoscience on trying to take concepts "from chemistry to circuit". On the materials science side, their focus centers on possible spintronic, magnetic, optical, or electrical nano-scale elements. These inorganic, building blocks for nanoelectronics have features fit for future circuits and could make interesting devices if used properly. Quantum dots have tunable optical properties. Carbon nanotubes are strong, and electrically and thermally conductive. Nanowires can be made of different semiconductors can be doped just like silicon. Even graphene and nano-magnets have interesting repercussions for waveguide and spintronics research. However, as we will see in Chapter 2, these nanomaterials with pertinent electrical, optical, and magnetic properties are still limited by their manufacturability. Self-organization, or more often referred to as self-assembly in this paper, does not come naturally to most of these materials of key interest. The communities spear-heading research have not identified techniques that would allow others to organize those sub-

elements into a greater circuit. And this is after at least 5 years of research for each of these materials. At the forefront of nanoelectronics, many research groups have taken the aforementioned elements and made simple prototypes of devices in laboratories. Still, these demonstrations do not come with techniques that could add complexity.

This thesis explores the possibility that it is not the lack of novel research devices that undermines possibilities, but the lack of ways to add complexity, on the large-scale. And to add complexity, self-assembly is the key. From a development standpoint, there are a number of things that cannot be done in a scalable way, on the large scale, with nano-scale precision. For example, we still need a scalable way of making the metal contacts to the array of devices, on the nano-scale. It is difficult to do that without resorting to the aforementioned non-scalable technologies. Carbon nanotubes never graduated as silicon technology replacements because no one ever figured out to mass-produce and mass-manipulate them in a low cost way. Using the examples we elaborate on, we entertain the idea that inorganic-based materials intrinsically simply do not lend themselves to adding complexity. This could be why techniques to make even small circuits out of inorganic sub-elements do not exist yet. But regardless of the reason, to go beyond the science of these sub-elements, we also need to think about what sort of techniques can make a circuit out of the smaller sub-elements. Doing *that* would go beyond nanoscience and give hope to future nano*technology*.

Techniques to build larger functional systems out of single molecules exist in biology, but implementing biological working principles and machinery for electronic, optical, etc. use has inherent challenges. If we take electrical properties for instance, the construction of functional electronic circuits from *just* biological molecules is not possible. This is because the majority of biomolecules exhibit poor electronic properties: they are mostly insulators. The good news with electrical properties is that the conductivity of bio-related materials is actually changeable. So organometallic materials, which *can* be self-assembled, definitely fit our definition of a material that lends itself to complexity (can be self-assembled) and works (is conductive).

In pursuit of a material that innately lends itself to the ability to add complexity, the discussion of what makes a material ideal is begun in the first two chapters. But the hard part is achieving a compromise that gives us all the benefits of our ideal solution. After temporarily assuming DNA is a perfect material, we see why it could be. But why it is not leads to Chapter 5's discussion of still more alternative materials. We will touch on electronic transport through these biological, but non-electronic, molecules as we get into the details about the choice of material.

C. Higher level view of our end-goal

There has been substantial progress towards inventing a next-generation technology that follows in the footsteps of silicon-based technology. With the impending problems implied with the push to continually achieve Moore's Law using the current device architecture, nanoelectronics is in search of new storage and processing technologies. So far, we have always presumed that the most basic unit of information processing will basically work like a tap: with electrons flowing through source and drain, controlled by the gate. This variety of devices, both mainstream and alternative, have been anywhere between current and voltage-controlled. To make such devices, some technologists are utilizing inorganic materials, such as carbon nanotubes, in hopes that these technologies would eventually be more compatible with the current silicon-based technologies as research progresses. Others are looking into using organic molecules to make an active device work like a switch when placed in the channel.

In the spirit of divide and conquer, many "single-disciplinary" approaches have led to partial solutions of the miniaturization problem. Researchers in chemistry and materials science have found ways to make nanostructures, such as quantum dots floating in solution of various shapes. Physicists use mathematical modeling of electron transport to uncover the electrical properties of various nano-objects (i.e. graphene, benzene), with supportive experimental evidence provided by experimental physicists. On the other end of the problem, electrical engineers try to improve materials, such as the insulating dielectric under the gate, or operate transistors under the threshold voltage in hopes of directly affecting the power wasted by today's high-density circuits. Solutions currently used by industry have come from physics and materials science. Focusing on parts of the problem has led to positive development and successfully held off the inevitable end of Moore's Law. But in an effort to go beyond the single disciplines and really fuse them for the sake of adding complexity, I propose the use of biological principles proven to benefit nanoelectronics.

So while the single-disciplinary approach to the greater problem has helped, a multi- and interdisciplinary approach is necessary. Such a synergistic approach could create a solution that works on all levels. For example, simply making the channel operate correctly in the nano-regime would not meet the needs of a technology developer. The ability to add complexity is of pivotal importance. But very few studies are on how to add complexity to new, alternative devices, be they made from organic molecules or semiconductor or carbonaceous nanomaterials. Some interdisciplinary work on a variety of organic molecules, borrowed from biotechnology, analyzes their optical, electrical, etc. characteristics. Certainly, the study of the electronics of such sub-units is fundamental to the purpose of nanoelectronics. But to transcend

nanoscience, we need to develop flexible mechanisms to organize and bring together these sub-units in a feasible way. And before bothering about behavior, we need to figure out *what* the nature of such elements would be, and *how* we can organize all these elements to make a system of electronic devices. To understand biological self-assembly, it is important that we introduce ourselves to new principles of chemistry.

First I brainstormed about what sort of material would be optimal for the purposes of nanoelectronics, keeping complexity in sight. Let me go into two key characteristics of the perfect self-assembled material, as per our requirements as electrical engineers. We want a sort of material that can be arranged in a way that complexity and organization could help build active, passive, or connective units.

In the most general way, in nanoelectronics, we seek a material or molecule that it can be organizes itself. It would be ideal if this material had a diverse toolkit (an diverse set of associated techniques that could be used for nano-manipulation) that fits our needs. Potentially, this material could double as a nanowire, or it could be converted into n-type or p-type material if methods of nano-manipulation exist. This functional unit may not be act as a device independently, but its conductivity properties will be important nonetheless. We need a type of material that gives us the ability to program or place one end of it here and one end of it there. In Chapter 2 we will discuss materials that cannot be biologically self-assembled and in Chapter 4 and 5 we will discuss materials that can be biologically self-assembled. Each are valuable and each have limitations, which all will be discussed in the respective chapters. One possible ideal characteristic for a self-assembling material is whether it can function as *nanowire*.

The importance of having a template for organization is that such a template would allow us to add complexity or organization to self-assembled active, passive, or connective units. We could assign locations to certain nanostructures and make a two-dimensional or three-dimensional structure with electronic, magnetic, optical, or mechanical functionality. Parts could function as active units, some as passive units, and the rest would either act as mechanical struts or electrical connections. Optimally, these elements could be positioned using a type of self-assembly similar to what biology uses. It would be best if such a nano-scaffold were electrically insulating since it would act the way an oxide does in silicon-based technology. Another possible ideal characteristic for a self-assembling material is whether it can be used as a *scaffold*, for positioning and organizing other electrically important materials.

Let me demonstrate how self-assembled materials exhibiting either functionality could benefit on a large-scale. If we had nanowires that could be self-assembled at our disposal, then

we would be able to assign it two ends and tell the wire to grow from here to there. If we had a scaffold that could be self-assembled, we could assign quantum dots to go attach to every periodic location, in a 2-dimensional or a 3-dimensional space. Using this same approach to attach multiply types of sub-elements, we could build an array of devices. Certainly, just being able to self-assemble the scaffold is not sufficient. We would also need a compatible technique of attaching different conducting elements. But by using scaffolds of preexisting, self-assembling nanomaterials, we might be able to better solve the problem of complexity with these new biological principles, based on a new brand of chemistry, previously unrelated to electrical engineering.

Now that we have brainstormed what characterizes the perfect nano-material, this opens up the table for discussing where we can look for the perfect material. For Chapter 2 of this thesis, let us assume that we do not need a material that has a biological self-assembly aspect. I survey the array of possible inorganic materials that could be used as a nano-wire. In searching for the value and limitations of each of these technologies and their supportive toolkits, we see the sort of need that exists for biological principles, to add complexity.

Biological self-assembly, enabled by the chemistry described in Chapter 3, can be employed by nanoelectronics, given we start with the right material. Biologically or chemically self-assembled molecules can come with tools like proteins for DNA, which allow engineers to manipulate and organize these nano-objects at the nano-scale using chemistry. To use are currently being investigated at a large scale. For example, technologists specializing in DNA have found ways to fold DNA into complex structures that may or may not be useful for our purpose: Chapter 4 discusses DNA scaffolds of this sort. What's enviable about their technique is the extent to which they can manipulate DNA using enzymes and proteins in solution.

Once we understand the benefits of using DNA, we can ask ourselves whether we want to use DNA as a nano-wire or for a scaffold or both? If we only want to use DNA as a molecular wire, then the conductivity of DNA comes into play, and as we will see in the beginning of Chapter 5, DNA is not a good conductor. But this could work for us if we only want to use DNA to organize other electronically functional nano-things, such as quantum dots. Because then, we want DNA to behave like an insulator, like a uniform oxide. Eventually, in Chapter 6, I will explain how both may be possible by selectively controlling the conductivity of DNA. Thus, DNA may be used as both the nanowire *and* the scaffold. But before jumping to this conclusion, this paper will walk you through the possibilities that lay before us.

D. A Glimpse at the end-goal

A number of things need to work together in order for "the future" to happen. But say everything worked together to give an optimal solution. What would "the future" of this technology look like? Nucleic acid-based electronics has the potential to enable a handful of interesting, niche technologies.

If DNA is assumed to be the material of choice, one possible vision involves three-dimensional DNA circuits. Already, DNA scaffolds exist (see Chapter 4), and this idea would use the scaffold to our advantage. I can use this concept to explain two ways of using the scaffold: (1) placing nano-elements and nano-devices using it, or even (2) using the scaffold as the basis for making interconnects and devices. The first possibility assumes the working devices and interconnects would be made of non-DNA material, such that the scaffold simply gets used for device position or guidelines for the wires. The second possibility is that the devices are made from the scaffold, using the scaffold itself to turn into something that works like a device. Here, the devices could be made out of the unit cells of the scaffolds and the interconnects could be made out of the lines of DNA extending from each device. Using either technique, you would have a circuit made of devices that are vertically and horizontally aligned in three-dimensional space. A two-dimensional layer of devices wired together could even be wired to a layer of devices above it. This could help improve the density of devices achievable on a single chip: of course, this DNA-based chip would be much different from a silicon-based integrated circuit. Note also that in a DNA scaffold, that is three-dimensional, it is much easier to make a large vast array of devices. Hopefully this kind of circuit could be self-assembled: in other words come together in a way that is chemically natural, or chemically becoming of the material. If the technique to make such circuits is not too labor-intensive and time-intensive, this would increase its promise as a technology.

A DNA scaffold could also be used to make optically functional layers: here is an example of a future solar cell using quantum dots. DNA strands can weave back and forth on each other to form little flat rafts. Such molecularly flat layers of DNA need to be characterized optically for this application. And once the characterization has taken place, we can employ methods to fold those two-dimensional rafts over one another to make groups of DNA layers. Now, that's just a solid of DNA scaffold material. Regular arrays of quantum dots can be positioned on to each of those layers of DNA. Quantum dots with different sizes have different absorption properties: the properties of quantum dots are *tunable*. Specifically, laws of physics reveal that small quantum dots absorb higher energy-wavelengths (blues and violets) and larger quantum dots absorb lower energy-wavelengths (oranges and reds). If DNA layers with smaller

quantum dots precede DNA layers with incrementally smaller quantum dots, then every layer could capture a specific range of wavelengths of light, thereby optimizing the solar cell design. Again, self-assembly would make this a more achievable and accessible technology for use by the photovoltaics industry. The use of the quantum dots in DNA layers also has implications for the creation of novel nano-capacitors.

Perhaps the most straightforward application for DNA-based devices would be for use as bio-probes. Silicon-based nano-probes would be unlikely because silicon is not biocompatible: on the other hand, bio-probes using DNA-based devices or circuits could be handy otherwise. Nano-scale pathogens, cell machinery, or other molecular things might be detectable via small changes in existing molecular machinery. Incorporating such modified bio-molecular machinery so that they work in concert with DNA-based electrical devices could enable bio-probes. These probes could detect mutations of different sorts, be used to check if certain functionalities were still working properly in the cell. If the circuit itself was a memory element that somehow indicates a state after detection, retrieval could give insight into cellular conditions. This sort of bio-probes could also be used to do minimal diagnostics for areas of the body that are dangerous to perform surgery on.

Thus, we see how involving electrical, optical, or any type of device with DNA and scaffolds made of it really opens up many technological possibilities. A lot of biology and chemistry goes into building those DNA-based scaffolds we spoke of before. But before we dive into the biology and chemistry, let's take a look at what we have in problems and solutions in existing materials science literature. Because the possibility that we can just align nano-elements on to the scaffold itself still exists. So after looking into existing materials science literature, we will gauge what we need, and the characteristics of an optimal solution.

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CHAPTER II.

Inorganic approaches & their limitations

This thesis aims to come up with a way of spatially organizing and wiring together nano-scale elements. So in this chapter, we begin with a study of possible candidates for nano-scale elements in our ideal, next-generation circuit. Since I am not in pursuit of inventing new sub-elements for circuits, we start off by analyzing existing sub-elements. By means of a literature review, we survey the spectrum of inorganic "semi-solutions" for materials that could be used in a next-generation transistor, or transistor replacement. In my "chemistry to circuit" approach to nanoelectronics, I narrow the scope of this chapter down by the type of nano-elements we are looking at.

We look at carbon nanotubes and silicon nanowires and analyze whether either would make suitable nanowires in our ideal, next-generation circuit. We start off with the assumption that a *suitable* nano-element exists for the purposes of nanoelectronics, and challenge that assumption by analysis. We will first look into their physical and electrical properties, and the techniques used to fabricate them. As we will see, each of these technologies has an associated toolkit—a number of things that you can do with them at the nano-scale. But how do we define *suitability*? (1) Does the material have device potential? (2) Is there a way to organize them en masse or is there a way they could self-assemble? Analyzing the peripheral research will reveal whether the sub-element has device potential. And investigating the proposed applications of these sub-elements will show if their toolkits lend to adding complexity.

We will see a theme arise here: the ability to efficiently add complexity is usually an issue for these inorganic sub-elements. And eventually, we see how our assumption (that we have a suitable nanowire for achieving a circuit) gets disproved. The use of "anti-complexity" technologies cannot be circumvented in the laboratory.

The innate chemistry of these materials do not provide a way of mass manipulating or self-assembling them. Through the discussion of the properties of carbon nanotubes and silicon nanowires, we see how, without embracing biological principles, none of these nano-elements can be a suitable nano-element by our definition. And this is despite their optimal electronic, spintronics, etc. properties. But before we depart from inorganic "semi-solutions," we explore its value.

A. Carbon nanotubes (CNTs)

Carbon nanotube (CNT) research has been on going since the early 1950s, making it the oldest area of nanowire research. In 1952, the Russians published the paper marking the beginning of CNT research, with the discovery of a graphitic 50 nm-diameter material [5]. The 1960s and 1970s oversaw the steady discovery of the novel characteristics of these fullerenes. Scientific findings began to snowball in the late 1980s and 90s, replete with publications that developed fabrication procedures. Although researchers extensively characterized CNTs over this time, concrete ties to silicon-based technology remained hard to achieve.

Physically, CNTs are graphitic sheets rolled into seamless cylinders, and can come in two varieties: single-walled or multi-walled. As the name suggests, multi-walled nanotubes contain multiple, cylindrical, concentric layers of tubular graphene. For our discussion, we focus on the properties and potential of single-walled nanotubes. Two defining physical characteristics for single-walled nanotubes are their length, their diameter (usually in nanometers), and their chirality. The length of nanotubes can extend up to 1.85×10^8 nm: that is, 18.3 cm long (these can be made using a specially tailored form of chemical vapor deposition[6]). Diameters can range from 0.5-100 nm, with the extreme cases coming from the inner and outer layers of multi-walled nanotubes. Typical single-walled nanotubes exhibit diameters of about 1 nm. Chirality can be described as the angle in which the graphene sheet is rolled to "make" the nanotube, denoted by (n,m) values. For example, armchair and zigzag nanotubes (see Figure 1a & 1b) have (n,m) indices n = m. The Young's modulus (around 1 TPa) [7] and tensile strength (13-90 GPa) [7] of CNTs make them ideal candidates for a scaffold material. But because they exhibit optimal electrical properties, using them for scaffolds would defeat their possible use as an inert scaffolding material.

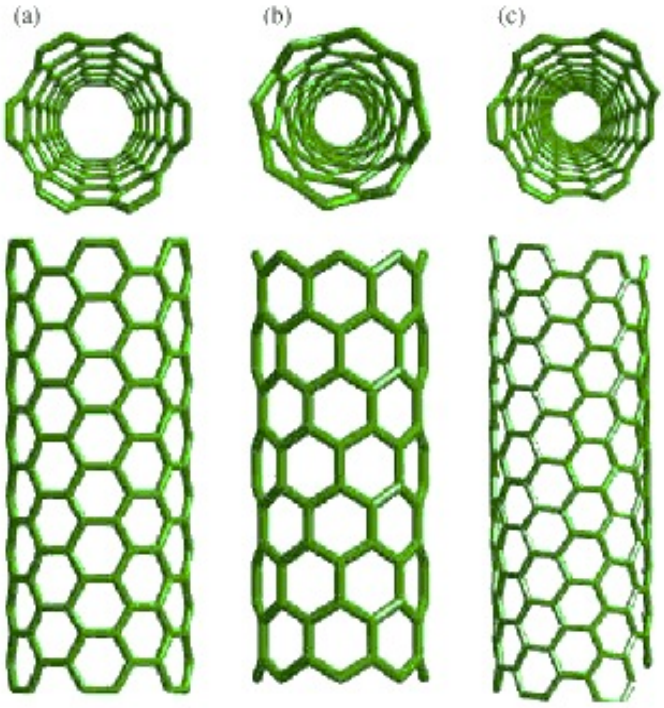

Figure 1. Types of carbon nanotubes: (a) armchair, (b) zigzag, (c) chiral.

As far as electrical properties go, carrier transport in nanotubes can be described using one-dimensional, ballistic transport models*. Beyond that, the type of electrical conduction depends on the chirality of the carbon nanotube exhibits. Armchair and zig-zag CNTs are metallic (see Figure 1a, Figure 1b, respectively), and chiral CNTs (see Figure 1c) show semiconducting properties [8]. If n – m gives a multiple of 3, then the resulting nanotube behaves like a semiconductor with a small band-gap[8]. Other chiral nanotubes exhibit moderate band-gap, with the band-gap's width depending inversely on the tube's diameter [[8]]. Theoretically, the metallic nanotubes can carry electrical current densities up to 4×10^9 A/cm^2 (1000 times greater than current densities of copper and other metals). Carbon nanotubes exhibit carrier mobilities of around 10,000 cm^2/V·s at room temperature, so they compare well with doped silicon [9]. Because the surface carbon atoms have satisfied orbitals, CNTs need not be passivated like silicon [10]. All of these electrical properties speak to device potential of carbon nanotubes.

Due to many promising electrical characteristics, CNTs are suitable for devices that operate in high frequencies. And for years, researchers have tried to make a device comparable to the silicon-based field-effect transistor. But the carbon nanotube-based field effect transistors (CNTFETs) that have been demonstrated in literature rely heavily on "anti-complexity" technologies [[10]]. The model CNTFETs that do not rely on such technologies do not exceed silicon-based transistors in operation. For example, one CNTFET channels made using the random web-like overlap of CNTs left by air-drying suspension fluids of CNTs. Hence, available fabrication methods define how achievable these potential devices are.

The synthesis techniques for creating carbon nanotubes are arc-discharge, laser ablation, and chemical vapor deposition (CVD). *Arc-discharge* uses two graphitic rods as electrodes in an inert gas environment (i.e. helium or argon) during arc-discharge. This method produces a random mixture of multi-walled and single-walled nanotubes, etc. Existing controllable fabrication parameters does not result in the growth a particular type of nanotube, so post-synthesis purification procedures become necessary. *Laser ablation* involves intense laser pulses ablating a carbonaceous target containing 0.5 atomic weight percent of nickel and cobalt. The metals attach to the fullerenes and prevent closure, facilitating growth [11]. Tri- and bi-metallic catalysts metals are more productive than single metals. Larger diameter CNTs are produced at higher temperatures. With either of the aforementioned processes, high temperatures can be used to make high-quality carbon nanotubes. Both arc-discharge and the laser ablation method are very cheap ways of making CNTs. For CVD, a substrate with a layer

* For more information, look into Peierls' Theorem

of catalytic metal nanoparticles that serve as the stems for carbon nanotubes. After heating the substrate to around 700°C, a process gas (i.e. H_2, N_2, or NH_3) and a carbon-containing gas (i.e. ethanol, methanol, acetylene, or ethylene) are bled together. As the carbon-containing molecules break apart and become the carbon atoms of the CNTs. Doing this in the presence of strong electrical fields results in more directionally aligned CNTs. The advantage of CVD is that no purification is needed and the CNTs can be grown directly on the substrate of choice. This process is the preferred method of commercial CNT production. An adjusted form of CVD may be used to produce 95-99% semiconducting CNTs [12]. But the advantages of these methods are not what limit the future of CNTs: it's the disadvantages of these methods.

Both the arc-discharge method and the laser-ablation method are hard to control: they grow carbon nanotubes in highly tangled forms, with carbon-based and metal impurities. The parameters available for adjustment in each of these methods cannot not selectively make metallic versus semiconducting nanotubes. Methods for post-synthesis, high-yield purification includes column chromatography but these methods often complicate how CNTs can be organized into circuits on a large scale. For example solubilizing CNTs and using functional groups to orient them using an electric field or something else ruins their electrical properties [13]. And although CVD does not require purification, it is expensive compared to arc discharge and laser ablation. And all these disadvantages translate to significant hurdles between nanotubes and their suitability and practicality for use in nanoelectronic devices.

A major obstacle to realizing nanotube-based devices is the lack of technology for mass-production. Also, as CNTs have structurally sensitive electronic properties, the available flexibility in the laboratory synthesis techniques is not enough. The knobs of the arc-discharge and laser ablation method do not translate to meaningful selections of carbon nanotube types. For CVD-grown CNTs, a method for zapping and electrically burning out metallic CNTs from mixture of CNTs exists [14]. But there is no way to remove the semiconducting ones and leave the metallic ones intact. Even with the use of nanotube bundles, it is necessary to take the statistical average of the constituent CNTs' properties. Basically, with any of these nanotube production methods, controlling conductivity is difficult.

Another issue is physically positioning nanotubes in way that is highly efficient and accurate on the nano-scale. Because the perfectly aligned growth of carbon nanotubes is not reproducible, researchers have to rely on "anti-complexity" technologies to physically position them. But even the automated manipulation of CNTs (a top-down approach) would not resolve the need for efficient bottom-up engineering in nanoelectronics. So even though the electrical

properties of carbon nanotubes are great, there is no way to position them and add complexity. These two drawbacks currently cripple CNTs ability to significantly impact nanoelectronics.

Nevertheless, researchers have made singular laboratory demonstrations of CNTFETs. The IV-characteristics of these proof-of-concept devices do not exhibit industrial grade reproducibility or robustness. More importantly, their techniques depend on "anti-complexity" technologies and their methods rarely exhibit ways to add complexity. So while the chemistry of CNTs is well understood, the current electrical engineering toolkit does not contain the tools to make multiple devices and circuits out of CNTs.

B. Silicon nanowires (NWs)

Silicon nanowires are thin solid cylinders of silicon material with diameters ranging from 1-100 nm. Nanowires of this sort can also be made with metals (such as nickel, platinum, and gold), with other semiconducting materials (like indium phosphate and gallium nitride), or even with insulating materials (silicon dioxide, for example). Researchers can fabricate nanowires using top-down approaches like lithography and electrophoresis. Bottom-up fabrication techniques include suspension, Vapor-Liquid-Solid (VLS) growth, and solution-phase synthesis. To create electronically active elements, nanowires can be doped too, using specific conditions of VLS growth. Scanning Electron Microscopes can be used to weld together nanowires as small as 10 nm in diameter.

Nanowires are effectively one-dimensional materials, and this strongly governs their electrical properties and related device ideas. Quantum confinement manifests itself in discrete values of electrical conductance. Edge effects dominate in the theoretical models of electron transport. Dr. Charlie Lieber of Harvard University and his laboratory pioneer the device possibilities of nanowire-based devices. A working device based on semiconductor nanowires would require two specific nanowires to cross another nanowire, without touching (see Figure 2). As long as the two nanowires are not touching, this device could potentially be voltage-controlled or current-regulated. For example, in the voltage-controlled version, a saturation voltage could be achieved above a certain gate voltage. But like CNTs, the precision of fabrication techniques of nanowires limits the mass-production of such a field-effect transistor.

Like carbon nanotube fabrication, reproducible arrays of aligned nanowires are difficult to engineer. Methods for positioning these nanowires post-production usually rely on "anti-complexity" technologies for applications that require accuracy. The device idea of the cross-wire transistor is special in how it uses a nano-element to achieve electrical functionality. It is also laudable that researchers have demonstrated working laboratory-scale proof of the

concept. But there is not a technique where this device can be mass manipulated on the nano-scale. Also, without a way to add complexity, these ideas are unsuitable for use beyond the laboratory environment. Arrays of FPGAs have been created with roughly aligned nanowires, but they still have no way of adding interconnects to each device. Again, the major hurdle to realizing nanowire-based devices, too, is the lack of technology for mass-production.

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The aforementioned nano-elements (and their analogs) might provide the eventual replacement for conventional MOSFETs. But clearly, moving out of the laboratory environment into the production line requires radically different thinking. Laboratory demonstrations encounter *huge* problems when it comes to organizing more complex circuits. So further research that achieves the efficient addition of complexity is crucial.

Taking a step back, we see that, basically, the assumption that we have suitable nano-elements is unfounded, and there is a pattern in the limitations of nanowires and nanotubes. Generally, given high resolution, throughput is often sacrificed to the extent that complexity cannot yet be achieved. Besides CNTs and nanowires, experimental physicists have tested still other atomically regular nano-objects, such as fullerenes and graphene for electrical conductivity. But few have been able to effectively tackle the problem of finding an upper-level technique for organizing nanomaterials.

Now that we have discussed nano-elements and their chemistry, I want to suggest how we can make circuits. We will organize these constituents: they may be nanowires, carbon nanotubes, or whatever nanomaterials prove pertinent. The organization scheme will not only have to position or wire together the device units but also position the elements within the individual devices. It would be a simpler task if devices were supplied as complete units somehow but, as we saw, no known research group has done such work. But there is a way biological principles might get us there.

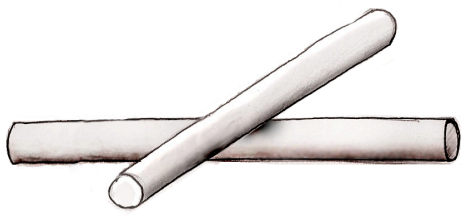


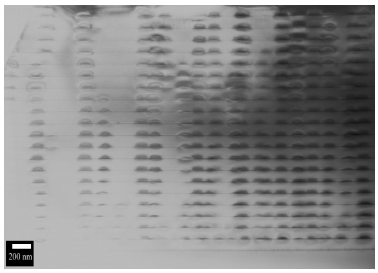
Figure 2. NW cross-wire field effect transistor: in theory, two nanowires perpendicular to each other and not touching (to prevent a short circuit) could behave like a field-effect transistor. Carbon nanotubes in this configuration should behave similarly. But to date, there are no means of positioning two nano-elements *and* maintaining an appreciable distance between them on a large-scale.

C. Partial solutions: inorganic self-assembly and molecular electronics

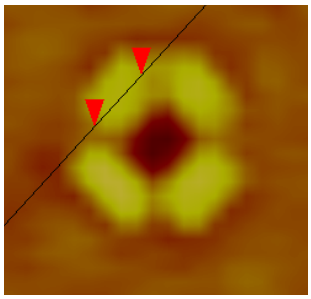
Ultimately, the issue with most of the aforementioned technologies is the difficulty in mass-producing them. And in *biology*, we see the ultimate examples of the mass production and organization of molecules. Many of these nano-elements described in the preceding section require "anti-complexity" technologies for positioning them. And before we discuss organizing, we needed to know what we were going to organize, so that was our first stepping-stone. What limits the mass production of silicon-based or inorganic molecular objects at the nano-scale does not limit biological self-assembly [15]. Biological chemistry is our second stepping-stone, because if biology's methods could be harnessed for the sake of electrical engineering, then we could achieve a truly multidisciplinary approach. But going between, bridging, & unifying silicon-based and nucleic acid-based electronics requires a basis in chemistry. Chemistry reveals why it is so necessary to consider technologies based on biological self-assembly.

Ideally, technologies derived from or closely related to more *existing* silicon-based technologies could bridge the gap to large-scale self-assembly. Phenomena similar to crystal growth, condensation, nucleation, or phase separation may inspire other techniques. But this family of techniques, which we refer to as inorganic self-assembly, has already been explored in recent years. The recurring theme with this research is that inorganic self-assembly, at best, sometimes offers a means of *short-range* ordering. An example of the products of such research is exemplified in the TEM and AFM micrographs of stacked GeSi islands (see Figure 3a) or the self-assembled quantum cellular automata-like structures (see Figure 3b). Such processes used to provide short-range order might help assemble carbon nanotubes or silicon nanowires. But because the forces involved weaken when applied on a larger scale, ordering breaks down (see Figure 3c). It follows that we cannot stop at inorganic self-assembly.

The products of molecular electronics research also lie at the interface of organic and inorganic "semi-solutions". Molecular electronics concentrates on a variety of organic molecules that exhibit switching behavior at a given voltage. Their robustness of their functionality depends on the integrity of device construction. To construct a device, a single, dense monolayer of these molecules is deposited on a substrate. Atop the deposited monolayer, a conductive layer is applied to complete the device. If even one molecule is missing in the monolayer, depositing the second conducting layer may result in a short circuit. Thus, the density of the monolayer is crucial to the functionality of the device. Herein lies the Achilles Heel of molecular electronics: lack of molecular accuracy compromises the functionality of the potential device.



(a)



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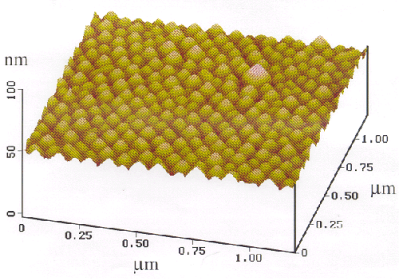

(c)

Figure 3: Examples of how short range ordering can be accomplished with inorganic self-assembly. (a) TEM micrograph of stacked GeSi islands growth by Stranski-Krastanov method, a type of epitaxial growth [16]. (b) AFM micrograph of a group of 4 GeSi islands for use as QCA, grown using MBE. The distance between the two red arrows is ~1 nm [17]. (c) AFM micrograph of large-scale application of MBE [18].

If we are going to do this, we need to do it right and we need to go all the way. This chapter bears witness to our access to electrically appropriate nano-elements that have device potential. At least with the case of carbon nanotubes, we need a way to control conductivity with precision. To help position these nano-elements, perhaps an electrical inactive nano-scaffold would be appropriate. With respect to that scaffold, we would also need a way to mass-manipulate these nano-elements. Ultimately, we want to be able to position them with single-molecule accuracy. We just saw how inorganic self-assembly does not give us a way to do all of this. And molecular electronics also falters at the point of single-molecule accuracy. Thus, we now turn to the source of large-scale self-assembly: biology.

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Picture references:

- Carbon nanotubes (http://mrsec.wisc.edu/Edetc/nanoquest/carbon/index.html)

CHAPTER III.

An Organic Approach: Self-assembly & DNA

The techniques described in the previous chapter showed how inorganic chemistry could be harnessed to make electronically functional pieces, wires, and some possible devices. To go beyond that, this chapter explains how biological self-assembly offers a more feasible way of adding complexity to the aforementioned technologies. With these biological principles in hand, assembling nano-elements, devices, and circuits may become possible via true bottom-up engineering. Potentially we will be able to rig this technique to not only make devices out of sub-elements, but also make circuits out of devices. Biological self-assembly can enable the formation of a better interdisciplinary solution for nanoelectronics.

Two basic examples can illustrate how biological principles can benefit nanoelectronics. Dielectrophoresis, for example, is a method used in biology labs, where a dielectric particle (like a cell or a protein) is subjected to a non-uniform electric field. Using a dielectrophoresis-based method, an array of 70-nm nanowire-graphene devices can be made [19]. Dielectrophoresis method is a self-limiting process allowing assembly of only one single nanowire on each pair of electrodes with high yield by controlling the hydrodynamic and electric field forces. These devices exhibited a high cutoff frequency approaching the theoretical limits of graphene. Although this method exhibited a way of positioning an array of devices on the nano-scale, it did not mention interconnects. A MIT-UCLA collaboration resulted in a method of using a cylindrical virus as a template for self-assembling of carbon nanotubes [20]. A four-step process attached the carbon nanotubes to the protein shells, and then wrapped the virus/SWNT complex in TiO_2 (see Figure 4). Incorporating semiconducting CNTs increased electron diffusion length, but decreased when metallic CNTs were included. The effectiveness of the final product depended on how they controlled the type of carbon

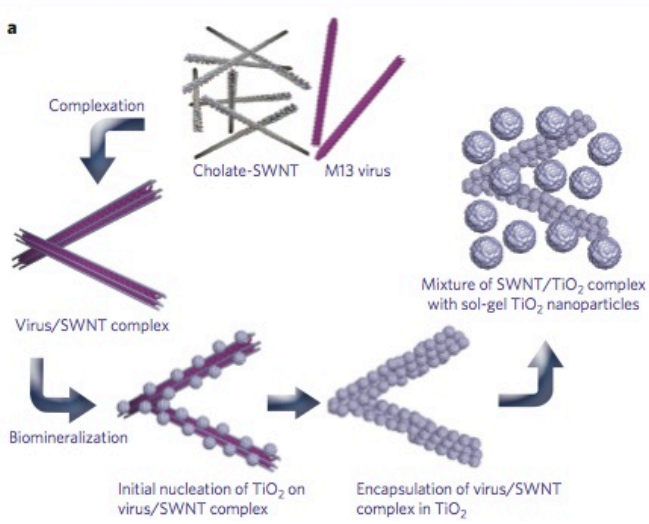


Figure 4: Method of self-assembling virus-based bundles of single-walled nanotube (SWNT) bundles for use in photovoltaics.[20] This technique shows how simple using biological self-assembly can be. Its simplicity and molecular accuracy fits our needs.

nanotubes. Granted these are also partial solutions that do not attack the aforementioned problems full on, but they are a start. As such, these examples demonstrate how bigger and better things may be possible with biological principles, in terms of circuits.

In our first section, we define how biological self-assembly is fundamentally different from inorganic self-assembly. Biological self-assembly radically departs from inorganic self-assembly because it is a different sense of mass production. Different molecular forces come into play and using the right tools favors the long-range ordering of nano-objects. In our second section, we lay down the fundamentals of DNA, as it is the prime example of self-assembly. Our third section, we survey the proteins that can work on DNA and all of the things we can do with DNA. The following chapters will discuss how we could harness all these things and techniques for the cause of nanoelectronics.

A. What do we mean by "self-assembly"?

How is biological self-assembly even relevant to nanoelectronics? As we saw earlier, inorganic self-assembly does not address the need for complexity. On the opposite end, biological self-assembly **makes** a variety of functional organisms possible. The way it works handles complexity, albeit a different kind, elegantly. In a sense, biological complexity is similar to what we need. Because the sort of complexity biology deals with, it allows biological self-assembly to order molecules specifically and accurately: something we need. Organisms are vastly more complex than the electronic devices of molecular electronics, let alone any of the singular devices achieved in the laboratory for nanoelectronics.

Evolution has adapted biological self-assembly to effectively deal with complexity by using molecules as packets of information. The self-assembly of programmed materials (to make nano-shapes and such), to DNA- and virus-based scaffolding of nanoparticles, exhibits everything this system is capable of. Biological self-assembly has a significant advantage over inorganic self-assembly that makes it work on the large-scale. In an organism, molecules spontaneously form ordered aggregates and in ways that involve little to no human intervention. All it requires is the correct chemical environment. Protein folding, the formation of nucleic acid structures, and macromolecules such as the ribosome are all examples of biological self-assembly [21]. In the last decade, the tools of molecular biology have developed to the extent that their findings are now accessible for interdisciplinary applications outside of biology [22]. Although biology is far from creating an electronic organism, enough is known to harness the framework of various subsystems to realize non-biological functionality at the molecular level. For example, enzymes (molecular catalysts of the body) can be purified to carry out reactions in

test tube-conditions. Proteins and enzymes can even be tailored to a variety of needs: computer-aided design of proteins has helped engineer complex properties of a high-ordered nano-protein assembly [23]. These recent developments in molecular biology make biological self-assembly available for use in nanoelectronics.

Two key factors separate biological self-assembly from inorganic self-assembly [[22]]. In biology, information is encoded molecularly and shape governs function. For example, the order of nucleo-bases (A, C, G, T in DNA) or amino acids encodes information. Sometimes this information is encoded by shape, i.e. by how a protein is folded. So a folding protein or a piece of DNA contains information about its activity in way that inanimate solid materials (whether they are atoms, nano-particles, or other nano-objects) do not. These types of macromolecules are better described as aperiodic crystals. The second thing, distinguishing one from the other, is how molecules become the blueprint for other molecular organization. In living matter, organization comes about by means of a pre-existing genetic code. In a sense, we get "order from order". A genetic "map" of genes tells the organism what to make, when to make it. To make the greater system work, biology depends on (1) the reliable transfer of information across generations, and (2) molecular machines [[22]]. Since DNA is at the center of how both of those things, the rest of the chapter will cover DNA.

In biology, there are numerous macromolecules with repeating units, but nucleic acids are unique to our purpose. Nucleic acids have an edge because the ones used by nature already have a pre-existing toolkit of proteins. A protein is another type of macromolecule found in cells, which is made of amino acids linked together by peptide bonds in a specific sequence. These proteins can be purified and used to change and manipulate that macromolecule outside of the body, in the lab, in test tube conditions. Also, nucleic acids can even be synthesized chemically! So on the subject of biochemistry, DNA is as good a starting place as any. Granted other nucleic acids exist as well, like RNA and PNA (discussed later). But so much is already known about DNA, and there even exists a lot of work tying DNA to nanoelectronics (discussed in Chapter 5 & 6). Also, in the environment, DNA is more chemically stable than proteins. Understanding DNA, and the microcosm around it, will thoroughly illustrate what successful biological self-assembly involves.

Admittedly, this is a rough explanation of DNA and biological self-assembly, so for more details, please resort to the references cited. Here, we are going into only the relevant concepts proved useful to our purpose by papers cited in later chapters. By covering DNA, we cull what biology has to offer, rather than trying to synthesize building blocks from primary principles.

B. DNA core concepts

DNA is a double-stranded macromolecule, found in the nucleus of practically every cell of an organism. Each strand is made of repeating units called nucleotides. Each nucleotide consists of a sugar, a phosphate group, and a base, popularly known as A, G, C, T (see **Figure 5**). "DNA" stands for deoxyribonucleic acid. Literally breaking down this name, gives the structure of the nucleotide. "Ribo" refers the 5-membered carbon-oxygen ring (ribose, a sugar), and "deoxy" indicates that the sugar has one less –OH group hanging off of the carbon chain than usual (the orange hydrogen, in **Figure 5**, replaces that –OH group). The phosphate is linked to the sugar via an ester bond (carbon-oxygen single bond) and the sugar is attached to the base via a glycosidic bond (carbon-nitrogen single bond from a sugar). These bonds come into play when we start talking about how proteins "recognize" DNA. When we see the numbers 3' and 5', these numbers are referring to the carbons of the sugar in the nucleotide. Numbering the carbons starts with the carbon that the base is attached to, and the second carbon is where the –OH group has been taken off. The charged phosphate groups, which come off of the 5' carbon, help stabilize the structure of DNA *in vivo* (in the body, in a living organism), via Coulombic repulsion. But, the net negative charge on the phosphate group means that DNA molecules *are not electrically neutral*. This plays a big role in determining the conductivity of DNA, discussed in Chapter 5.

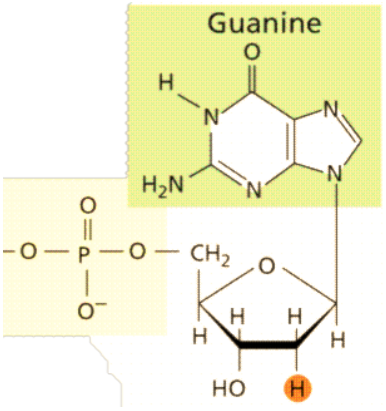


Figure 5: The structure of a DNA nucleotide. The part highlighted in yellow is the phosphate group and the part highlighted in green is the nitrogenous base. It could be any base, but this nucleotide has guanine stuck on it for example. The un-colored carbon-oxygen ring at the bottom is the sugar.

The order of nitrogenous bases characterizes any given strand of DNA, so let's figure out what biologists mean by "bases" and "base pairs". The base can have a double-ring structure (purines) or single-ring structure (pyrimidines), and involves nitrogen and oxygen atoms. The names of the bases are adenine (A), guanine (G) (both purines), cytosine (C), and thymine (T) (both pyrimidines). Thermodynamically, it is more favorable for adenine to pair with thymine, and guanine to pair with cytosine. Three hydrogen bonds form between guanine and cytosine, and two hydrogen bonds form between adenine and thymine. Together, we call these "base pairs" (bp): the number of base pairs often implies the length of a strand of DNA in a sequence or strand. The order of bp makes up the "primary" structure of DNA. So although the genetic code has 4 "bits" to play with, so to say, because they pair up consistently, it is kind of like a 2 "bit" system as well. When biologists "sequence" DNA, they are figuring out the order of

the bases along a strand. We will cover the structure and bonding between base pairs in more detail in Chapter 5.

Zooming out of the molecular viewpoint, let us look at why the DNA takes on this "secondary" structure, or 3D configuration. This structure is mostly the result of the molecular interactions between adjacent base pairs. The interior of the DNA structure, the base pair stack, is actually pretty hydrophobic. If any water molecules were involved in the stack of base pairs, it would probably mess up the hydrogen bonding between the base pairs. In fact, a van der Waals attraction between base pairs allows them to come in close contact with each other. What results is a stack of base pairs, one on top of the other, with barely any space between them. Usually these interactions are termed *stacking interactions*: they are the main force holding the two strands together in a helix [24]. This makes the structure of DNA in solution quite stable.

A physical description of the shape of a DNA molecule gives us an idea of sizes relative to the inorganic nano-elements we spoke of in Chapter 2. Usually the structure of the DNA can be generalized as two strands of backbone (the sugar and phosphate groups) and coordinated base pairs. Biological DNA takes on the formation of a right-handed helix, like a ladder that curls up. As the helix twists up, the planar base pairs sit like rungs of a ladder along the twist, spaced 0.33 nm apart. The helix turns about 34.6° per base pair.[24] On an average, 10.4 base pairs span each revolution of the twist, a total of 3.40 nm (this is approximate, as the actual number depends slightly on what the base pair sequence is) [[24]].

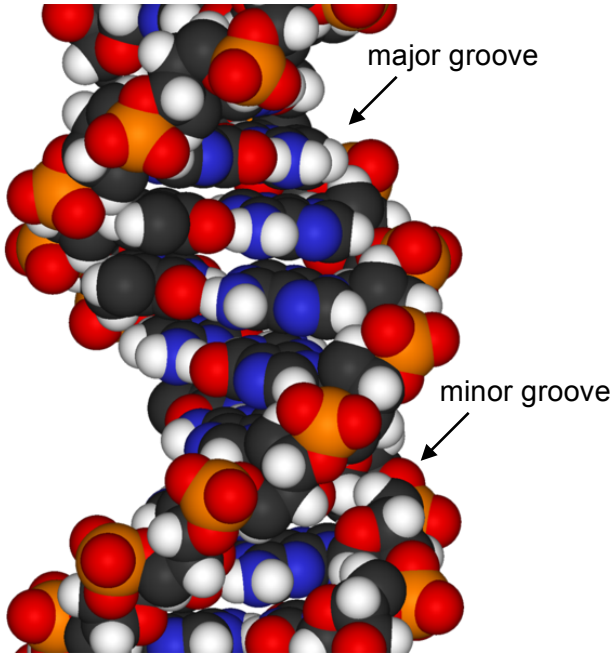


Figure 6: Space-filled model of DNA's double-helix structure. White represents hydrogen, dark grey is carbon, blue is nitrogen, orange is phosphate, and red and oxygen. This figure shows the major and minor grooves in the helix. Note how the bases are more accessible in the major groove.

The helix gives DNA a type of periodicity, despite all the complicated molecules within the actual chemical structure. In the double helix, the strands of the backbone are not all evenly spaced. The two strands that are attached to each other via the bases are typically closer to each other. This creates a major and minor groove in the overall structure of a DNA double helix (see **Figure 6**). The width of the DNA spiral, including the major and minor groove, is 2.37 nm. The major groove is 2.2 nm long, revealing the bases a little more than the minor groove, which is 1.2 nm long. In solution, the base pairs touch water along the major groove. The major

and minor grooves will come into play when we cover proteins and how they access the base pairs.

As for the mechanical properties of DNA, literature studies have been done on the material properties of DNA in solution. In an aqueous solution, DNA has the ability to bend, twist, and compress with the double helix structure. DNA copes with thermal vibration and collisions with water molecules by slightly changing conformation. So because it does not have a perfectly stationary and rigid structure, the classical measurement of its rigidity is impossible. Nonetheless the definition of "persistence length" can be tweaked for DNA. Assume we define it as, "the length of DNA over which the time-averaged orientation of the polymer becomes uncorrelated by a factor of *e*" [25]. According to this definition, the persistence length of DNA is 46-50 nm (about 140-150 bp). This means DNA is pretty flexible. At the same time, chromosomal DNA has a Young's modulus of 0.3-1 GPa (akin to a brittle, hard plastic). In polymer physics, the *Kratky-Porod* worm-like chain model represents DNA quite accurately. Anyhow, these mechanical properties make it a favorable choice for a nano-scaffolding material.

In the world of nucleic acids, the chemistry of DNA is a good mix between stable and flexible. Out of all the self-assembled molecules in the body, DNA is more stable compared to RNA. Note that in biology, and especially biochemistry, the word "stable" typically refers to macromolecules that enzymes and proteins cannot "break down" [26]. And proteins are not the only things that can alter DNA or any given macromolecule: light, heat, other sources of energy can also change or mutate DNA. So let us discuss different ways DNA can chemically or thermodynamically "break down".

When we think of DNA, our minds classically visualize its native structure: double-stranded DNA (abbreviated dsDNA from here on out) with the double helix and base pairs all connected correctly. But at temperatures just above normal body temperature, thermal energy undoes the double-stranded structure. In biology, when the DNA helix comes undone, it is called denaturation. The hydrogen bonding between the base pairs is the first to go, exposing the inner bases. Once divided, the 2 separated, spiral molecules left with their bases revealed are called single-stranded DNA (ssDNA). Denaturation temperature depends on molecule length and base sequence (in particular, GC content because GC bonding is stronger than AT bonding) [27]. But once a strand is made, the energy required to split that dsDNA into two ssDNAs is constant, since the bp sequence is not really changing. This is important because DNA-modifying proteins need that amount of energy to access the inner base pairs.

The remaining advantages of using DNA remain in the number of things we can do with DNA. Very accurate molecular manipulation of DNA is possible via DNA-binding proteins.

C. DNA-binding proteins and applications

DNA is a molecule that is easy to chemically manipulate with the right proteins. Proteins are macromolecules that can act on DNA. Enzymes can too: enzymes are a type of protein that can catalyze a specific chemical reaction. Usually a specific biochemical event requires a high energy of activation, but specific enzymes can lower that activation energy barrier, facilitating that event or activity. Organisms have a plethora of proteins and enzymes that can work on DNA and RNA. Once we know how the proteins work, we will talk of DNA metabolism, that is, DNA replication, repair, and recombination.

But first of all, what are proteins made of? Amino acids are the building blocks of proteins: depending on which textbook you look at, there exist 20-22 standard amino acids, and even more nonstandard amino acids that are not natively found in proteins. All amino acids contain a carbon that links together a carboxylate ($-COO^-$) with an amino group ($-^+NH_3$). As carbon (in its uncharged state) makes a total four bonds, strings of different *functional groups*[†] can be attached to that carbon. Depending on what is attached to that "backbone" carbon, amino acids can be nonpolar, polar (uncharged), basic, or acidic. Acidity and basicity also refers to the charge of a molecule in biological conditions. Acidic functional groups have been deprotonated and are negatively charged within an organism and basic ones are usually protonated & positively charged. The amino acids are all linked via peptide bonds, a carbon-nitrogen bond made by making H_2O (with 2 H's from $-^+NH_3$ and 1 O from the $-COO^-$). A different sequence of amino acids characterizes every protein, and sequences are usually given from the N-terminus (the $-^+NH_3$ side) to the C-terminus (the $-COO^-$ side). Further molecular interactions between amino acids folds the string into a three-dimensional structure. This is called the protein's "secondary" and "tertiary" structure, giving them very specific nooks and crannies.

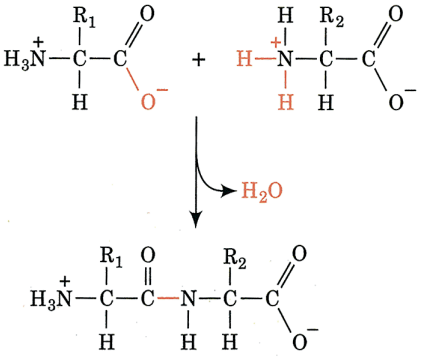


Figure 7: Formation of peptide bond between the carboxylate and the amino group. R_1 and R_2 represent the different things that distinguish one amino acid from another [26].

The location of certain amino acids is structurally meaningful because they offer the protein a high affinity for the molecule that's supposed to fit into it. You can think of proteins and enzymes like locks that can do more than just secure a molecule, and whatever fits inside of them is kind of like a molecular key. Biologists have an umbrella term for any molecule that binds to a specific site on a protein or other molecule: almost every protein has a *binding site*

[†] functional group - a specific group of atoms that form a "submolecule" so to say. These are motifs in organic chemistry. For example, –OH group is called "hydroxyl-" and –CH3 group is called "methyl-"

built for a specific *ligand*. The word "ligand" refers to whatever molecule or macromolecule the protein is meant to work on. The place or groove in the protein or enzyme that has a high affinity for a given ligand is termed the *binding site*. The types of intermolecular forces that prevail in living systems govern how ligands link up to a binding site. Polar covalent bonds are responsible for in creating permanent dipoles in proteins [26].

The permanent dipoles built into proteins and the shape of molecules give proteins a certain affinity to its ligand. You can think of the protein as having a pocket or dent, the way a lock that is shaped and charged exactly so that the ligand fits in like a key. *Each hydrogen bond* that forms between the protein and the ligand contributes to the affinity of that protein to its ligand. The lock-and-key model of proteins and ligands, and the strategically placed hydrogen bonds, explain how a protein "recognizes" its ligand. This is how shape governs function in biology. Evolution has designed naturally occurring proteins to have a high affinity for even a low concentration of it ligand. Evolution has also tailored each and every protein and enzyme to do its job with molecularly accurate enough for biological purposes. The greater number of hydrogen bonds or van der Waals bonds the ligand forms with its binding site, the more efficient an enzyme can be at its job.

Using these principles, DNA-binding proteins and enzymes attach to DNA in multiple ways. Proteins typically recognize DNA sequence via direct hydrogen bonding or van der Waals interactions with a certain part of the structure. This can happen in a sequence-specific (referring to the base pair sequence) way or a non-sequence-specific way. Sequence-independent protein binding generally involves ionic bonds [28] with the backbone. For example, these proteins will have DNA binding domains so the right amino acid "residues" can line up with the phosphate groups on the DNA backbone. Another common theme is that phosphodiester bonds form between DNA and either serine[‡] or tyrosine[§] residues in many proteins involved in DNA metabolism. The DNA proteins that only bind to ssDNA stabilize the single strand to prevent it from coiling onto itself. The majority of studies done on how proteins bind to DNA are conducted on sequence-specific binding. These proteins rely on "accessing" the base pairs by the major groove, bonding to them via hydrogen bonds.

When enzymes need chemical energy to do their job, they usually turn to a molecule called ATP (adenosine triphosphate). ATP is the energy currency in organic systems, and has two pyrophosphate bonds. ATP gets turned into ADP (adenosine diphosphate) when it is used once and AMP (adenosine monophosphate) when it is used twice. Many of these enzymes, and

[‡] a polar uncharged amino acid
[§] a hydrophobic amino acid

hence processes, are ATP-reliant [29]. For example, ATP concentrations regulate DNA replication. Certainly we will not be working *in vivo*, but many of the enzymes present in these systems need a standard cellular environment, to an extent. This guarantees the high efficiency of the macromolecules, in turning over substrate to product. So in order to use biological self-assembly, we will need to mimic the chemical conditions in which this or that takes place to get proteins to work properly in a test tube environment, also termed as *in vitro*.

DNA-binding proteins, and other proteins that work with DNA bind to it using the same principles. For now we turn our attention towards DNA-modifying enzymes, to see what the world of proteins has to offer, functionality-wise. DNA strands can be untwisted, separated, cut (in sequence-specific and non-sequence-specific ways), joined together, copied, and so much more using enzymes and proteins. Here, we go through what they do, how they do it.

"Ligases" and "nucleases" are the two types of enzymes that *rejoin* and *cut* DNA strands, respectively. The technical term for joining together two strands of ssDNA and dsDNA, or their linkage, is called "ligation". DNA ligases help form a covalent phosphodiester bond between the 3'-hydroxyl group and the 5' phosphate group. ATP helps catalyze the linkage between ssDNA or dsDNA 3' and 5' ends. DNA replication and repair depend on working ligases. On the other hand, how a given nuclease "decides" to cut a DNA strand can further classify the family of DNA nucleases. Some enzymes only cut close to the end of the strand (exonucleases), some only cut in in the middle of the strand (endonucleases), and some rely on finding recognition sequences (restriction enzyme) [^{26}]. Biologists use the word "cleaving" to describe cutting a DNA strand. Some nucleases are less useful because they randomly cleave along the length of the molecule. In 1970, scientists developed tools that could cleave DNA at specific sites, so DNA can be cleaved in predictable and reproducible ways [30], [31]. Molecular cloning and DNA fingerprinting make use of the sequence-specific nucleases. As far as modification goes, molecular biologists are more aware of the extent to which the molecular accuracy of these enzymes can be optimized.

Enzymes that depend on how coiled DNA is (to access the base pairs by the major groove) depend on the work of "topoisomerases" and "helicases". These two enzymes exhibit both cleaving and ligating capabilities, and can change how tightly DNA is twisted. The term "supercoiling" refers to how tightly DNA is twisted. Some topoisomerases cut the DNA helix, let one section untwist a little and reduce the amount of supercoiling, and then seal the break in dsDNA. Other topoisomerases cut one strand of the 2 present in DNA, then pass the other uncut strand through the break, and then seal the break. Hence, this uncoils dsDNA by one turn. DNA replication and transcription both need topoisomerases to get the job done.

Helicases are molecular motors that use ATP to break the H-bonds between the bases and unwind and unzip dsDNA into two strands of ssDNA.

Other enzymes include DNA modifying enzymes that can add or remove functional groups from DNA, and polymerases. In biology, this is important because, extra phosphate groups off the 5' ends of dsDNA indicate that either the DNA needs to be copied or that it has yet to be copied, for example. So "alkaline phosphatase" removes the extra phosphate groups to indicate the work there has been done (and so that copying does not restart). On the contrary, "polynucleotide kinase" adds phosphate groups back. More enzymes like this exist to adjust DNA chemically, in small, molecularly accurate ways. Polymerase is the enzymatic centerpiece to DNA replication, a process that requires a whole slew of helper proteins. So because DNA replication is complicated, the next section will cover how it copies DNA.

Perhaps out of all these protein "tools", restriction enzymes are the most useful: mostly, they are important for DNA fingerprinting. Over 3000 restriction enzymes known, and around 600 are available for commercial use. Let us see how DNA fingerprinting works. Say a given restriction enzyme cleaves DNA with the sequence CCCGGG exactly in half. In everybody, these locations would occur in different places. So after the restriction enzyme is applied to a given DNA sequence, each unique sample will have different amounts of differently sized DNA. Next comes the application of electrophoresis: this is what gives the "signature" of a person's DNA. Electrophoresis depends on the movement of charged particles in a fluid or gel under the influence of an electric field. This method effectively takes advantage of charge AND particle size; DNA has a negative charge. To begin electrophoretic analysis, samples (with the differently sized DNA segments) are placed on one end of the gel. Then, an electric field is applied, encouraging the differently sized DNA segments to drift through the gel at different speeds. Electrophoresis can also used to determine characterize and analyze proteins, using the same concepts. Thus, used in conjunction with electrophoresis, restriction enzymes can also be used to characterize a person's DNA.

Hopefully by now, you should have a good idea of how DNA proteins and enzymes work. Outside of a biological framework, it matters to us if all these proteins can be isolated or purified for our *in vitro* use. Not all proteins have been purified, isolated, and characterized. But because DNA is so important to biologists, most DNA-modifying and DNA-binding enzymes have or can be. Most of these enzymatic tools can also be modified via protein engineering. But Chapter 5 will discuss these options in more detail, so let's not get too ahead of ourselves.

D. Biological synthesis of DNA & more

Because DNA is so central to any given organism, evolutionary means of backing up and maintaining DNA also exist. For organisms, DNA transcription and translation are key parts of the process of DNA "expression". "DNA expression" refers to the order of operations that cells use to make proteins from the genetic code of DNA. But this does not really concern us: we are more interested in how the body makes DNA. The biological synthesis of DNA is part of DNA metabolism: DNA metabolism refers to the replication, repair, and recombination of DNA. In the body, the longest piece of DNA that occurs is part of Chromosome 1: typically it can be around 220 million bp long. It follows that when DNA is copied, it must be done with *extreme* accuracy. Life depends on this accuracy: mutated DNA in the most vital places of our genetic code can destroy an organism. The system of biological mechanisms that improve the accuracy of DNA copying is called DNA repair.

DNA Synthesis or DNA Replication

DNA synthesis is at the core of how awe-inspiring biological self-assembly can be. Each of the 2 strands that make up DNA is made up of a string of nucleotides (nt). Nucleotides are

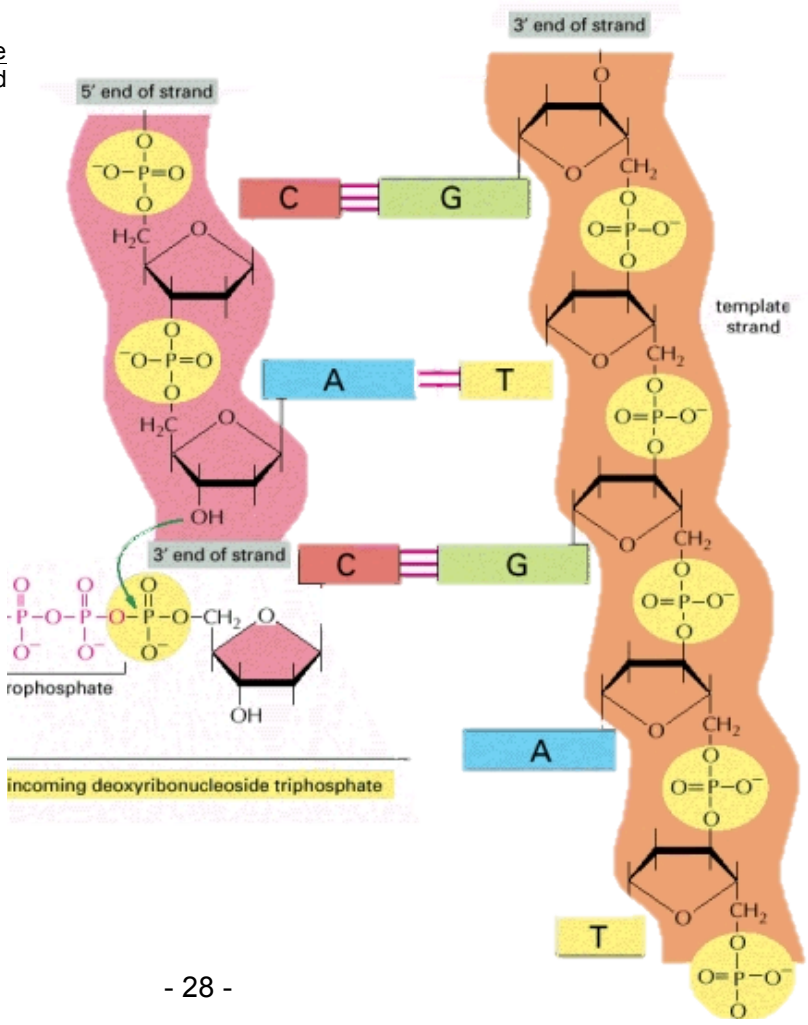


Figure 8: A protein-less view of the chemistry of DNA synthesis. The 3' end is where nucleotides are added.[26]

the basic building blocks of DNA, made up of a 2-deoxyribose (a sugar), a phosphate, and a base. Generally speaking, to copy DNA, the parent strand of dsDNA continuously unzips. Simultaneously, DNA polymerase adds new nucleotides to the template strands of ssDNA until you get two daughter strands of dsDNA. This is vaguely how the process of DNA replication works, and it occurs right where the two parent strands divide to form a fork. We know how topoisomerase untwists dsDNA, stabilizing proteins sit on the backbone to keep the strands singular, helicase provides the energy to unzip the base pairs, polymerase does the copying, and ligase can mend the backbone where necessary. And DNA polymerase is one of the best-characterized proteins out there, so its activity can be described pretty definitively. Let us see how all of these proteins work in concert.

Initially, the simplest mechanism of DNA replication seems to be the continuous addition of nucleotides to both sides [[26]]. But dsDNA has two strands of DNA that are "antiparallel". A single ssDNA always has one 3' end terminates on a 5' end. For DNA to twist and fit properly, the two strands have to go in opposite directions. One side goes 3'-to-5' and the other side goes 5'-to-3' (See Figure 8). For replication to occur by this "simplest" mechanism, proteins would need to have two different things going on. One side would make a new, complementary 3'-to-5' strand for the 5'-to-3' side, while the other protein would make a new 5'-to-3' strand for the 3'-to-5' side. Let us see how this is solved biologically.

At the replication fork, a multienzyme complex containing DNA polymerase adds DNA

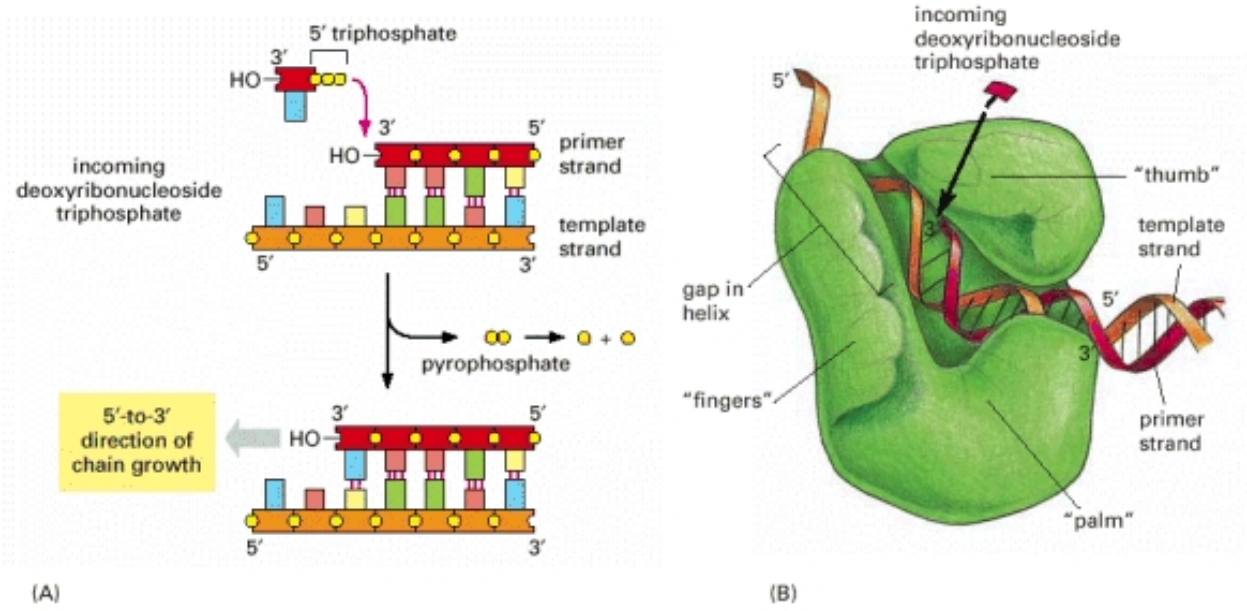


Figure 9: How DNA polymerase catalyzes DNA synthesis. The thermodynamic driving force, the release of a pyrophosphate group, is shown here.[26] For more information, see the video mentioned at the end of the chapter.

nucleotides. There is a separate nucleotide for *A*, *G*, *C*, and *T*. Think of this multienzyme complex like your hand, holding a string that gets pulled out from the thumb-side of your fist (see Figure 9). The polymerase is right where the first base of the parent ssDNA is. Thermal energy guarantees enough Brownian motion that we can wait for a nucleotide to fall into place pretty often. When a nucleotide falls into place, polymerase checks to see if it bonds correctly with the base on the parent strand. If it does, it catalyzes the addition of a DNA nucleotide to the 3′-OH end of the new polynucleotide chain. This mechanism allows the addition of 500 nucleotides per second, in the appropriate chemical environment. This means that addition is direct, linearly continuous on the 5'-to-3' direction (referring to the direction of the parent strand). The new strand on this side is called the "leading strand". All the types of DNA polymerase ever discovered can only work off the 3' side of the new strand [26].

So what happens on the other side? This is slightly more complex: it is called the "lagging strand" (see Figure 10). DNA synthesis is still occurring in the 5'-to-3' direction, but it has to wait for the other side to form a little bit before this side can kick in (see Figure 11). The synthesis of this side occurs discontinuously, as nucleotides are added, or polymerized. Because DNA is antiparallel, on this side, synthesis occurs in a direction opposite to overall DNA chain growth (see Figure 10). DNA ligase is absolutely necessary to stitch together these fragments of DNA once they are completed. These fragments are called "Okazaki fragments" and they usually range from 1,000-2,000 bp in size. By the time DNA polymerase on the lagging end runs into the complete dsDNA it made last time, there is some ssDNA waiting to be completed by it. Single-strand DNA-binding proteins polymerize on the loop of ssDNA, stabilizing it as it waits for polymerase to finish (see Figure 11). And when it finishes, DNA polymerase hops on over the new ssDNA and restarts the polymerization of nucleotides. In the meantime, DNA ligase completes the backbone of the lagging strand [26]. Note that although two flat, parallel lines are shown to represent DNA, these are cartoons. DNA actually takes on the double helix form once a complementary strand is present.

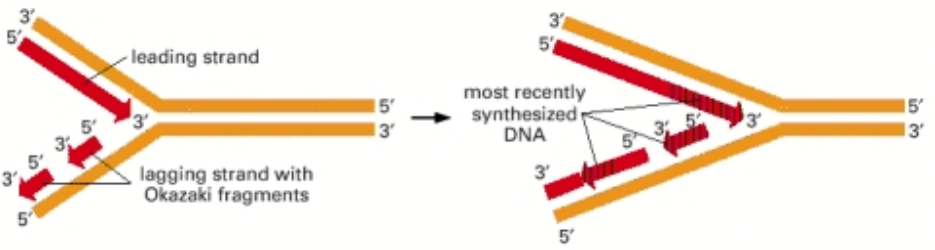


Figure 10: The structure of the DNA replication fork, showing the formation of the leading and lagging strands.[26]

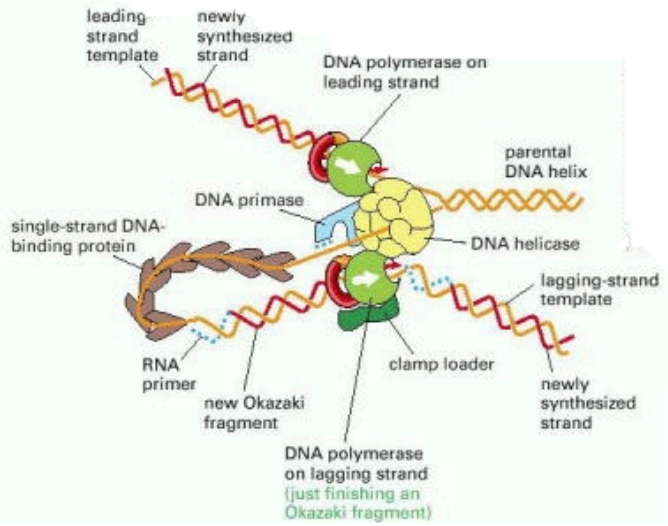


Figure 11: An active replication fork. This shows almost all the proteins involved in the process of making the leading and lagging strands.

This is how the proteins at the replication fork cooperate to form a replication machine. Hopefully, the above figures and the literal explanation clarified how all those molecules work in concert. In summary, Figure 11 shows a still-life version of what the whole system does, on both sides. But admittedly, if a picture can speak a thousand words, a video can speak volumes. Hence the actual mechanisms shown in the video links at the end of the chapter may help. To further understand DNA replication, please view the videos mentioned at the end of this chapter.

What happens when DNA polymerase adds the wrong nucleotide? The most accurate polymerases are part of a multienzyme complex that has a separate region dealing with that. First the incoming nucleotide forms hydrogen bonds with the parent strand's base: and wrong hydrogen bonds can be formed. Remember the fist analogy for DNA replication? This other region "tightens its fingers" around new pairing (see Figure 9). Because the correct base pairing is the most energetically favorable, this mechanism can recognize errors. After the erroneous nucleotide, a separate part of the multienzyme complex takes it from DNA polymerase before ligating the backbone. It promptly removes the incorrect nucleotide, rewinds the strand by one nucleotide, and gives the strand back to polymerase. This activity is called exonucleolytic proofreading [26]. In this way, the multienzyme complex that involves DNA polymerase is part of a "self-correcting" multienzyme.

Other less accurate DNA polymerases exist but they play only a small role in DNA repair, which we will discuss next.

DNA Repair

The most accurate DNA polymerases, which work first, make mistakes every 1 to 10,000 or 100,000 bp [26]. DNA repair mechanisms increase the accuracy of DNA replication, so it does not only depend on the initial base pairing. So, to invoke the full complexity of DNA self-assembly, *in vitro*, we may need to employ DNA repair enzymes appropriately.

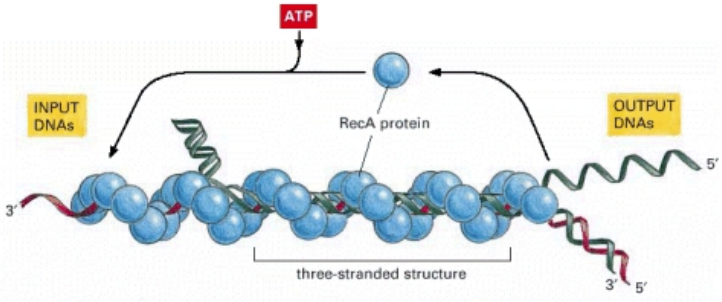
Because of the "limited" accuracy of DNA polymerase, a handful of "proofreading" mechanisms sequentially act on DNA to correct any mistakes. There are many ways DNA can be damaged: mismatches can occur and bases themselves can be damaged. To remove damaged bases or mismatches, enzymes called DNA glycosylases conduct base excision repair on DNA. These enzymes use ATP to flip out the bases and check to see if they are intact. For more difficult situations, when damage has resulted in a "bulky lesion," DNA helicase, polymerase and ligase help with nucleotide excision repair. This repair pathway first removes 12 bp of the messed up side, and then patches up the hole with polymerase and ligase. Additional proteins can reverse chemical damage in DNA. Outside of these, more repair pathways exist, including (1) enzymes that reseal dsDNA breaks, and (2) suboptimal polymerases that repair with less accuracy as biology's last-ditch effort. Less than 1 in 1,000 accidental base changes results in a permanent mutation, after DNA repair.

Homologous Recombination

There is even a way of shuffling genes in DNA: its called homologous recombination. Homologous recombination is where two DNA duplexes swap segments with similar nucleotide sequences. Swapping genes is important evolutionarily, resulting in the sorts of incremental changes that last from generation to generation. For the purpose of nanoelectronics, Chapter 6 will prove this method relevant to the solution of the greater problem.

The exact mechanism of how DNA wraps onto itself to swap genes is rather inconsequential for use in nanoelectronics. But there is a protein used in homologous recombination that has proved useful to researchers bridging the gap between biology and nanoelectronics. Recombination sites can be anywhere from 30 to 200 bp long. The protein RecA in *E. coli* can polymerize on ssDNA and stabilize a triple helix of DNA. But for the ssDNA to be incorporated into the dsDNA, it must have the same bp sequence as one of the strands in the dsDNA. This means that RecA, when used with a ssDNA probe, can bind to DNA in a *sequence-specific manner*. Thus, the sequence chosen for RecA to polymerize on, can behave like an address (see Figure 12). Biologists have been trying to figure out how this stuff exactly works because it is so important to evolution. But other than RecA, or other analogous proteins in other organisms, even the whole process of site-specific homologous recombination is not very useful for the purpose of nanoelectronics.

(a)



(b)

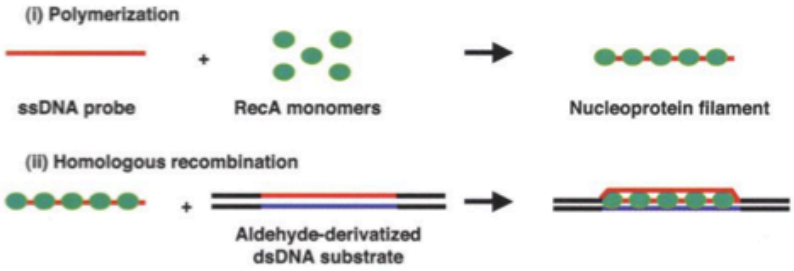


Figure 12: How RecA is sequence specific. (a) a cartoon showing how RecA facilitates a DNA triplex structure, via ATP concentration.[26] (b) how RecA can bind in a sequence specific manner.[49]

Holliday junctions are structures where two strands of DNA are being shared by still two other strands. This makes a planar junction between two dsDNA molecules (see Figure 13). DNA naturally forms this structure during homologous recombination, and the junction is free to move along the homologous region. An ATP-driven helicase typically stabilizes the junction and controls its movement. The way these junctions are made with two strands, junctions can also be made with three strands of DNA, which would enter the third dimension. We will see how this is useful in making nanostructures out of DNA in the following chapter.

In summary, DNA is *very* unlike carbon nanotubes, silicon nanowires, and other products of materials science research. DNA has hundreds of DNA-modifying enzymes, which allows researchers to manipulate DNA molecularly and accurately. Many of them can work on RNA as well (we will talk about other nucleic acids in Chapter 5). And these enzymes give us a way to manipulate them accurately and in reproducible ways on the molecular scale. Biological self-assembly shows how DNA can be synthesized from a parent strand. If we could use everything we know about DNA

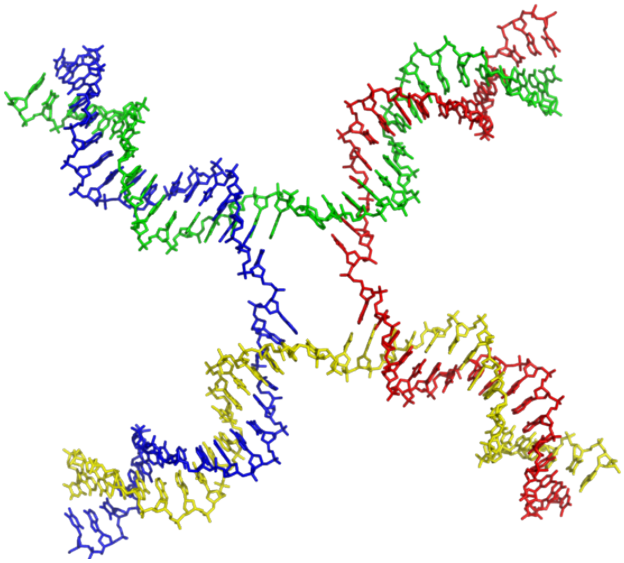


Figure 13: stick model of DNA strands in a Holliday junction.

to make nanostructures to position them accurately on the nano-scale? We would be all set—at least, with respect to the problem of positioning nano-elements in 3D space. Given the toolkit of proteins we just talked about, this seems possible. And Chapter 4 shows that indeed it has already been done. Using biological self-assembly could help position nano-elements on a large-scale and help form nano-scaffolds.

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Picture references:

- Nucleotide (http://www.emc.maricopa.edu/faculty/farabee/biobk/biobookdnamolgen.html)
- Space-filled model of DNA (http://chemistry.about.com/od/factsstructures/ig/Chemical-Structures---D/DNA-or-Deoxyribonucleic-Acid.htm)
- Holliday junction (http://en.wikipedia.org/wiki/Dna)

Pertinent videos:

- Self-correcting DNA polymerase: http://www.youtube.com/watch?v=TC2mYWR8754
- DNA replication showing the simultaneous formation of the leading and lagging strands (almost every protein is shown in the video, except for single-strand DNA-binding proteins): http://www.youtube.com/watch?v=bee6PWUgPo8

CHAPTER IV.

DNA Scaffolds and Nanostructures

Now that we know the gist of what biological self-assembly has to offer us, let us return to the challenges in nanoelectronics. There were two main challenges: (1) positioning nano-elements in a way that was precise at the nano-scale, and (2) maintaining control over conductivity. The purpose of this chapter is to exhibit how biological self-assembly impacts our solution to the problem of positioning nano-elements. Here, we explore existing research and work on DNA scaffolds and other nano-mechanical structures, with our problem solving hats on.

DNA nanotechnology is the field of research dedicated to the design and manufacture of artificial nucleic acid structures for technological uses. We are interested in making DNA scaffolds and other nanostructures because DNA is a form of *programmable matter*. We call it "programmable matter" because the base pairs of DNA can potentially let us "program" information about location into the molecule. Before we make the scaffold we can give each strand an address about where we want it to be in lattice we make.[32] But before we dive headlong into how to make scaffolds, let us take a step towards the chemistry we want to use to our benefit. What can we synthetically control, and what is our ideal situation with respect to DNA?

There would be a number of things we would want control over in DNA. We would want to control the base pair sequence for example. We would want to be able to make strands that are 10,000 to 100,000 base pairs long. Assuming that small devices, based on DNA segments of 10-20 bp, are possible, think of how long DNA would have to be to house an array hundreds or thousands of devices. On top of that, being able to interconnect them will also require more bp. This chapter will help answer these questions, through literature and using what we know is now possible with biological self-assembly. The key to our questions here is the "how?", so let me make a short analysis on the methods of synthesizing DNA.

Let us look into the chemical synthesis of DNA to see how much we really have control over in the laboratory. Whatever methods require us to tailor bp sequences will use chemical synthesis, which has limitations. Biological synthesis of DNA happens in the 5'-to-3' direction, but chemical synthesis happens in the 3'-to-5' direction. The building blocks in this case are either triphosphate nucleobases, or nucleotides with different protecting groups on them, depending on what method is used. The step-wise addition of single nucleotides is necessary to make a particular sequence. As the DNA chain grows increasingly longer, side reactions

increase the number of errors that occur in synthetic polymerization. This chemically limits the length that DNA can be made: synthetically, no more than 200 base pairs are practical. Limitations of this sort apply to even the best synthetic automated procedures out there. After the bp sequence of our choice is made, we can use PCR to amplify the strains we have. PCR is a test-tube version of DNA replication used to "amplify" (or, multiply the number of) small amounts of DNA: it is used in DNA fingerprinting.

So, in DNA nanotechnology, there are a total of about 2 practical options available. One is to completely engineer the bp sequence of DNA. And indeed, custom sequences (up to 100 bp long) are commercially available for use by laboratories. Typically if you want to make longer chains, ligating smaller segments together is more effective. To make really long chains via ligation, this process is still mostly done "by hand", chemically speaking. This is how gene synthesis makes strands up to 1,000 bp long, which is still an improvement. But beyond this, the lack of automation is also an "anti-complexity" problem in the biochemistry of DNA. So although, with chemical synthesis we have more control over the base pair sequence, the speed of nucleotide polymerization is compromised. The second alternative is to find a way to use random sequence stock DNA from the genes of bacteria (this is also commercially available, and typically cheaper). Analyzing different approaches to nano-scaffolding with DNA will help us gauge how to choose between these options.

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So here, we take a look into DNA nanotechnology for nanoelectronics. In particular we focus on how DNA and its toolkit can be used in the laboratory to make mechanical, molecular scaffolds.

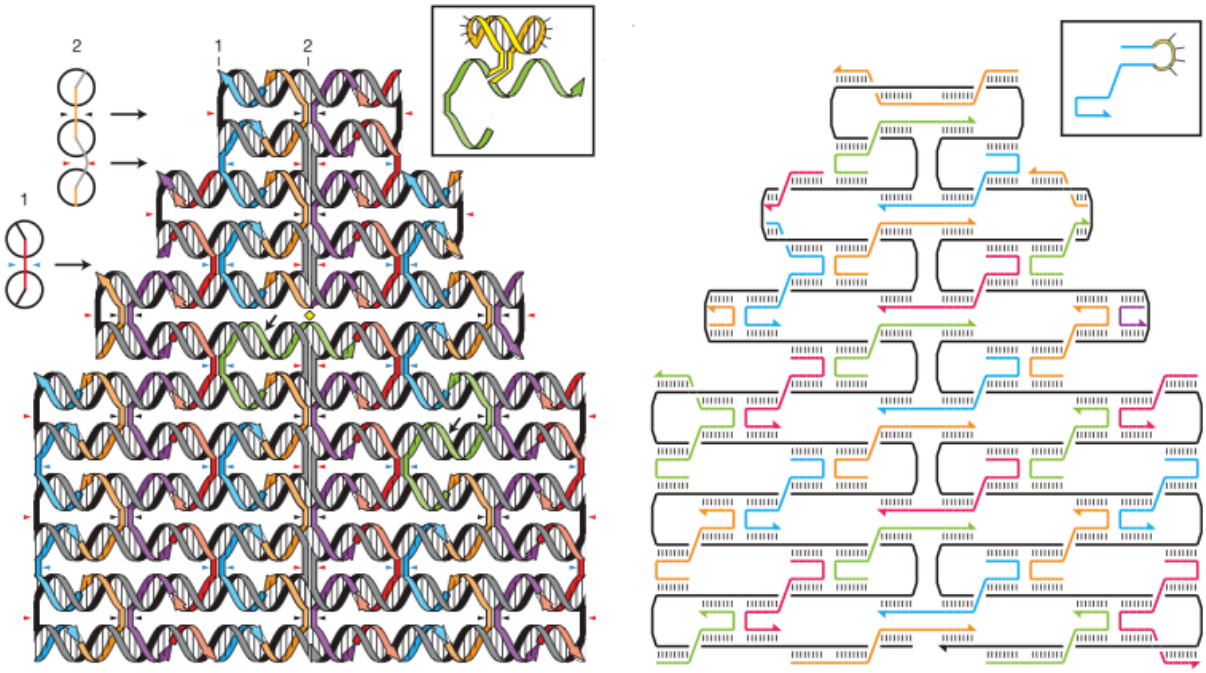
A. Nanostructures based on double cross-over DNA

Figure 14: A segment of double cross-over motif in DNA. It consists of 5 pieces of ssDNA that form 2 double helix domains, with two cross over points. Constraining each four arm junction to one direction, lends the molecule rigidity.

In 2006, Dr. P. K. Rothemund and his colleagues at Caltech reported a method for making nano-scale shapes and patterns using DNA. Rothemund refers to the planar products of his work "scaffolds". But this is not a nano-scale replica of what constitutes scaffolding in construction. Rothemund's planar nanostructures resemble the levels, or platforms of scaffolding surrounding a building more, without the struts in between. Rothemund's purpose is to defy the notion that in order to do DNA scaffolding, (1) optimized sequences, (2) highly purified strands, and (3) perfectly equimolar strand concentrations are necessary.

Figure 15: How DNA strands weave together to form two-dimensional nanoscaffolds. The thinnest lines represent bases. The yellow diamond shows where the staples can be cut and resealed to bridge the seam. (a) ssDNA strands are shown as helices. The ribbon's arrowheads show 3'-to-5' direction. This view also shows how many revolutions the DNA makes in each raster's progression. At the top of the structure shown, there are 3 turns, and at the bottom, a total of 9 turns. (b) Finished product where DNA is represented by cartoon lines, after appropriate merges and rearrangements are made along the seam. Most staples are 32 bp strands spanning 3 helices.

(a) (b)



The "double-crossover" (DX) motif is basis for Rothemund's method (see Figure 14). So let us see how ssDNA in this motif can be used to make two-dimensional "scaffolds" in the simplest way possible.

The sequence of base pairs is rather inconsequential to this method, so any stock viral or bacterial DNA suits Rothemund's purpose. The bp sequence of a long segment of this DNA (about ~7,000 of these bp) comes from virus M13mp18 DNA. This microbial DNA is, for all our purposes, completely random. As long as the 200 short staple strands match the sections of the longest ssDNA strand, it holds the scaffold in shape. These short strands are strategically placed to fold the long strand into whatever shape you want to program. Note, here it is necessary to take the "pitch" of DNA into account to make sure one strand can go to the neighboring strand in the right location. We went over this in Chapter 3 (page 20), but just as a reminder, the helix turns about 34.6°/bp, and about 10.4 bp span each revolution of the twist. Rothemund uses computer programs to calculate the locations of staple segments.

The tradeoff to not having to engineer bp sequences is that the DNA nanostructures must be rationally designed for the self-assembly of the desired structures. To favor the formation of nano-shapes, certain segments of ssDNA need to be complementary to the right

sections of other ssDNA. So weaving one piece of ssDNA with smaller ssDNA 'staple strands' makes the shape [33] (see Figure 15). The scaffold uses one long 7,000 bp-long piece of ssDNA that weaves back and forth to form double helices (the grey strand in Figure 15). Over 200 short oligonucleotide pieces chosen from the longer sequence are used to hold the shape in place (the colored strands in Figure 15). These staple strands are each around 30 bp long, and can be placed strategically to make almost any two-dimensional shape.

The biochemistry to make these nano-scale shapes and patterns involves a certain type of restriction enzymes and a temperature-dependent process. Rothemund began with the plasmid (another word for a type of DNA found in bacteria and other unicellular organism, which is typically circular) of M13mp18 virus (7,249 bp-long). To see if using 'staple segments' works to create a given shape, that pre-sequenced strand of ssDNA was chosen as basis for the scaffold. The BsrBI restriction enzyme removed the part of the plasmid that would mess up the weaving structure calculated to make a given shape, leaving 7,176 bp. Most designs were designed for less than 7,176 bp, so about 25 bp 'remainder strands' complemented the unused bit of the sequence. To make the shape, a 100-fold excess of 200–250 staple and remainder strands were mixed with scaffold and annealed from 95°C to 20°C for just under 2 hours [33]. The annealing allowed the DNA crossover helices to form properly, and create the nano-

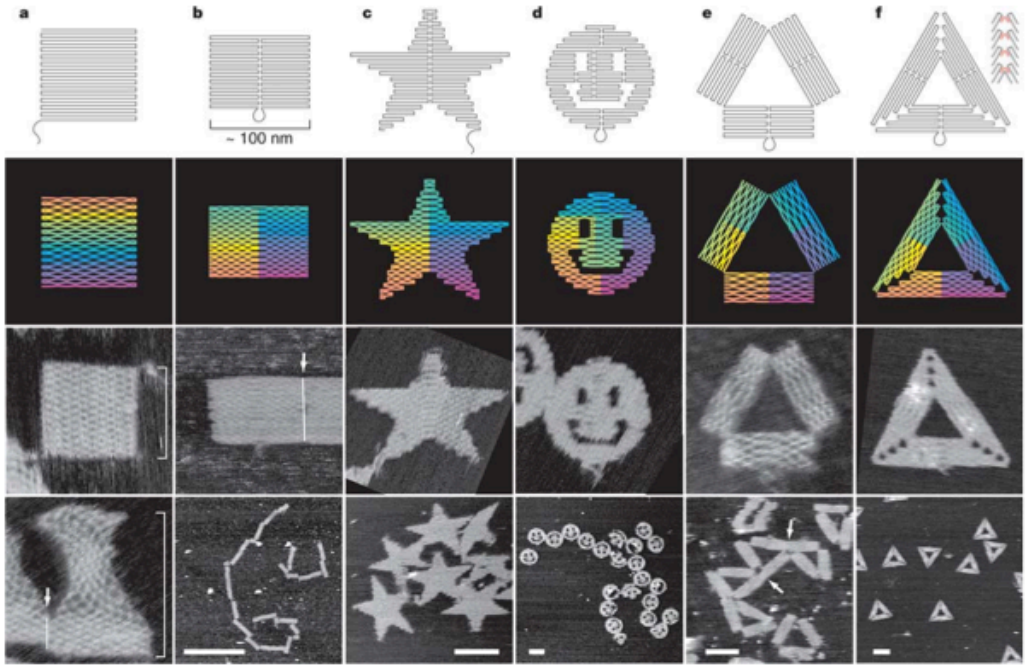


Figure 16: DNA orgami shapes. The first row shows folding paths. In the second row, the gradient of colors through the lines represents the beginning to the end of the strand. (a) square; (b) rectangle; (c) star; (d) a disc with three holes; (e) triangle with rectangular corners; (f) sharp triangle with trapezoidal corners (the little red lines indicate the use of ssDNA segments that bridged the three sides). All images and panels without scale bars are the same size, 165 nm x 165 nm. Scale bars for lower AFM images: (b), 1 μm; c–f, 100 nm.

shapes. After annealing, the samples were deposited on mica, and only folded DNA structures stuck to the surface. The excess staples, DNA strands and everything else remained in solution, so more purification proved unnecessary. Rothemund depends on AFM imaging under buffer to verify the creation of the shapes. Depending on how the staples are programmed, this method can successfully create six different nano-shapes. Figure 16 shows their predicted shapes and the AFM images of the “experimentally observed” DNA structures. He calls his method "scaffolded DNA origami".

The value of Rothemund’s work is its simplicity and its possible applications. The aforementioned means illustrate a way to make practically any two-dimensional shape using DNA origami. Each of the smiley face structures is about 100 nanometers across, 2 nanometers thick, and consists a total of approximately 14,000 bp. This technique can be used to make shapes with features that are even 6 nm in size: in a sense, this is the “resolution” of DNA origami of this sort. DNA origami patterned like this could be the basis of “nano-breadboards”, on which diverse components could be added. It may be possible to make nanoelectronic circuits on these by attaching nanowires, and other electrically functional nano-elements. Even three-dimensional structures are possible by this underlying technique, as the last section of this chapter will illustrate. All in all, it’s a good example of how bottom-up fabrication may achieve the specificity of top-down engineering.

Such nano-scaffolds can also help make three-dimensional shapes on the nano-scale. Dr. S. Douglas and his laboratory build on the fundamentals of Rothemund’s work, which resulted in sheets of DNA. Continuing this metaphor, the Douglas group depends on Coulombic stabilization to fold the sheets (more akin to the macroscopic concept of origami). More specifically, a variety of cationic species (namely NaCl, $MgCl_2$) accelerate proper folding [34].

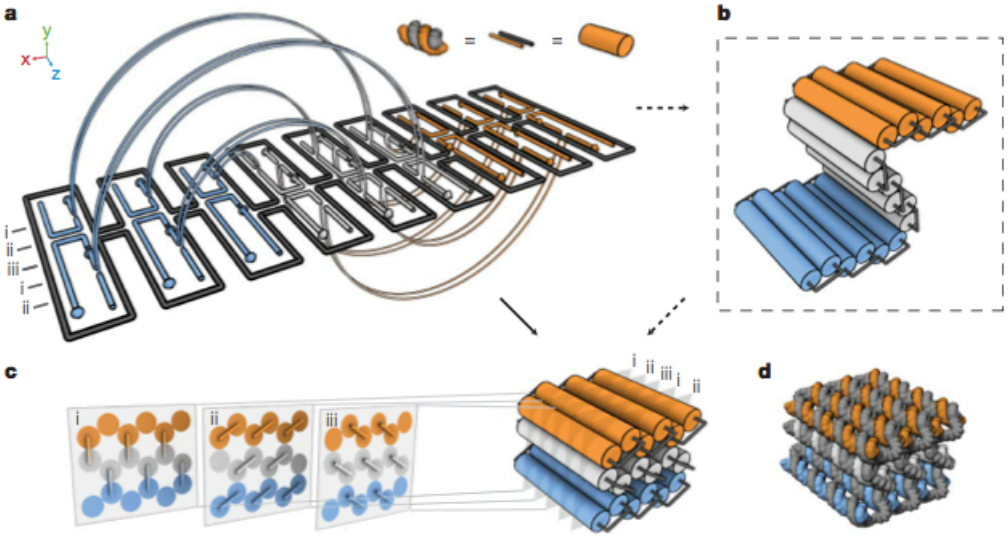


Figure 17: Design of 3D DNA origami, by Rothemund. Scaffold in grey, staple strands in orange, white, and blue. In (b), the cylinders represent dsDNA [34].

The folding results in DNA nano-logs, and a variety of other capsule-like nanostructures (see **Figure 17**). The Douglas Group uses staple strands to make the structures come together properly. Procedurally, that only requires more annealing steps, still little to no use of enzymes [34]. But since these annealing steps usually take days and weeks of time, improving the rate of these processes could really make this method more realistic and accessible. Applications in drug delivery and other biomedical uses appear to be on the top of Douglas group's agenda. But there might be ways to use Douglas lab's ideas for nanoelectronics.

But pertinent technical difficulties exist with Rothemund's standalone approach. DNA origami is synthesized in solution and deposition is hard to control: random arrangements are common (see **Figure 18**). This conversion makes it hard to characterize nano-devices built on these substrates or to integrate them with conventional integrated circuits. We will talk more about the technical difficulties of making this transition in Chapter 5. Rothemund's further attempts of integrating these nano-scaffolds with conventional integrated circuits involve EBL and AFMs (see **Figure 18**) [35]. Rothemund seems to run into the same problems that the researchers faced when they were trying to align nano-elements during growth. Also, an analogous way to make struts between levels in the third-dimension (like via's in ICs) would also help picture the addition of complexity. Thus, choosing "anti-complexity" technologies and top-down approaches to analyze and add complexity, yet again, limits the possibilities.

In a way, this sort of conclusion to such work defeats the purpose of using biological self-assembly. So future work for this approach may perhaps involve some way to add electrical functionality to the scaffold using biological self-assembly. This sort of development would be important in adding complexity and making interconnects. Note how Rothemund does not try to invoke the use of already-present molecular interactions or any type of pertinent chemistry (the type biological molecules are hard-wired to follow) to try to align the nano-

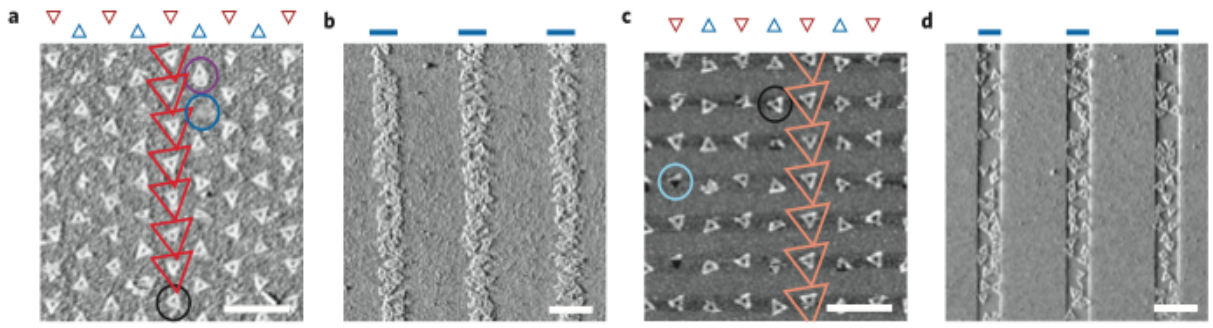


Figure 18: Difficulties of using EBL to orient the position of DNA origami shapes. AFM images (a) DNA triangles on ~110-nm patterned triangles on SiO_2 (red lines show oxidized patches); (b) DNA triangles on 300-nm patterned lines on SiO_2 show dense and random placement; (c) DNA triangles on ~110 nm triangles patterned on a diamond-like carbon (DLC)/DLC silicon surface; (d) DNA triangles on 200 nm EBL-patterned lines on DLC/DLC [35].

shapes. For example, proteins could technically still access the backbone of DNA on the edges of these structures. Proteins that could attach or connect electrically functional nano-elements or nano-devices would make this work more relevant to our purpose.

But before discussing full front-to-end solutions, let us delve into other ways of making nano-scaffolds.

B. Nano-scaffolds with Holliday junctions and sticky ends

Dr. N. Seeman at NYU focuses on how DNA can be used to make crystalline structures. He developed this method in which base pair sequence starts playing a bigger role. Seeman's group is well versed in the different ways of bringing DNA together and DNA's protein toolkit. His work is based primarily on two key features: (1) Holliday junctions, and (2) "sticky ends". We covered Holliday junctions at the end of Chapter 3. In organisms, Holliday junctions in DNA are typically mobile: since the bp sequence is complementary to both strands on both sides, they can zip and unzip each other as they please. To make the Holliday junctions immobile, a sort of asymmetry in the bp sequence needs to be introduced to force the Holliday junction to stay in one spot. As opposed to "blunt ended" DNA (where both strands are cut evenly at the ends which we have assumed so far), Seeman Lab makes extensive use of "sticky ended" DNA. "Sticky ends" are alternate extensions of one of two strands that make up dsDNA, used in genetic engineering. These ends have dangling bases whose sequence can be chosen to help us put that "end" where we want it "stick" (see **Figure 19**). The 3'-end and the 5'-end with complementary bases come together during annealing, as we saw in Rothemund's work. This is a way of using biological self-assembly to help position molecules accurately. For effective self-assembly, longer DNA molecules require longer sticky ends because increasing hydrogen bonding contributes to the two molecules' affinity for each other. In this section, we will see how the most basic application of these concepts results in flat nano-scale network of DNA strands.

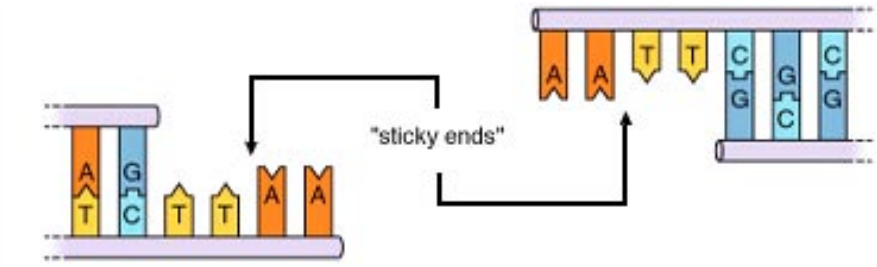


Figure 19: Sticky ends of DNA.

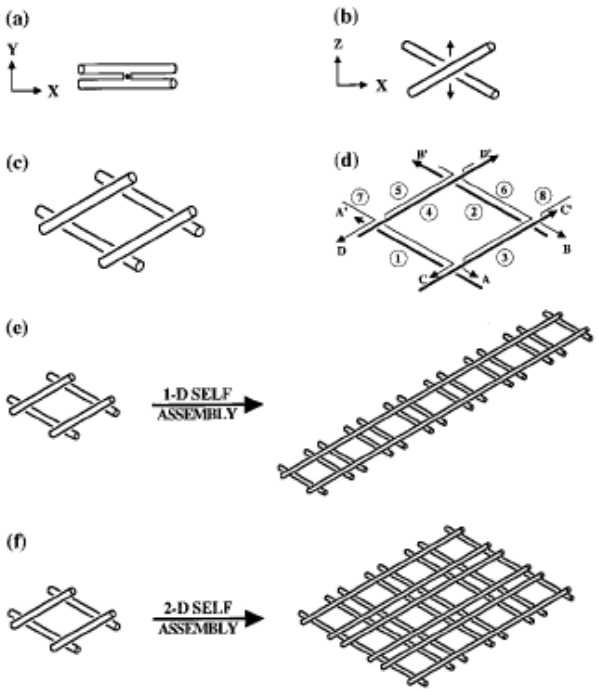


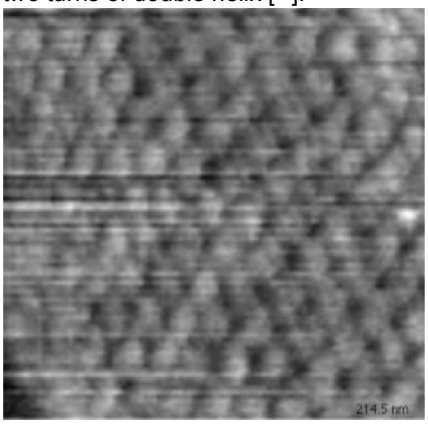
Figure 20: Schematic of Seeman groups illustration of how a DNA building block can form a 1D and 2D self-assembled DNA scaffold. For this diagram, cylinders represent DNA helices, and crossovers represent immobile Holliday junctions. (a) A view of a DNA Holliday junction from the side. (b) A view of a Holliday junction from above, showing the angle separation. For (a) and (b), the 2 strands are set 30° to each other. (c) Combining 4 Holliday junctions make a rhombus-like motif. At the junctions, the 4 strands are oriented like the structure in (b). There are six turns of DNA in each helix, and four turns between crossover points, and one-turn overhangs on the ends. (d) Arrowheads indicate the 3′ ends of the strands. Sticky ends are shown by the letters A, B, C, and D, and their complementary strands are A′, B′, C′, and D′, respectively. The molecule is constructed by synthesizing strands 1-8, with the selective use of blunt ends for the ends of the 1D or 2D structure. (e) How this motif could self-assembly into a 1D array. 1D self-assembly is shown to produce a railroad-track-like arrangement, with helices representing two "rails", extending for the length of the assembly, and "ties" separated 2 turns and 4 turns (alternating). (f) How this motif could self-assembly into a 2D array, with the same spacing as (e). Note that the latticework array contains two layers of DNA [36].

Seeman group demonstrated one-dimensional & two-dimensional nano-scaffolds, in 1999. Figure 20 shows the progression of how immobile Holliday junctions can make a building block nano-structure. Each of the cylinders crossing over each other represents an immobile Holliday junction. The resulting quadrilateral, despite having 4 legs of equally sized strands, is not a square per se. Because in solution, the immobile junctions still have torsional flexibility, so the paper refers to the "squares" as "rhombi" to be more accurate [36]. The legs of the building block made are made of 6 revolutions of DNA, or "turns" or cycles of the helix. Thus, each leg should be almost 21 nm, consisting of a little over 62 bp on each side, to account for the junctions and overhang. The DNA rhombi's cavities are tunable, although Seeman chose to illustrate 6x6 cycles building blocks and 6x8 cycles blocks too.

Seeman's lab engineers the base pair sequences using a program called SEQUIN. A DNA synthesizer makes the strands themselves, but the procedures beyond that are very biochemically-involved. For beginners, strand purification is crucial even after the first step. The published paper contains the bp sequences and a lot more procedural detail, involving particular ligases and exonucleases [36]. Hydrogen-bonded interactions condense the building block rhombi into periodic arrays. The strands need to be annealed at particular temperatures, be in the right chemical environment in the presence of enzymes, and have perfectly equimolar strand concentrations, amongst other conditions. For 1D self-assembly, the building blocks are adjusted such that each part only has sticky ends A and A', and B and B' [36]. For 2D self-assembly, selective use of the sticky ends, and having the correct molar concentration of each

of the pieces of the array is important. Making the "perfect situation" for biological self-assembly may be difficult, but Seeman's results are accurate enough (see **Figure 21**), and suggest that perhaps it is all is worth it.

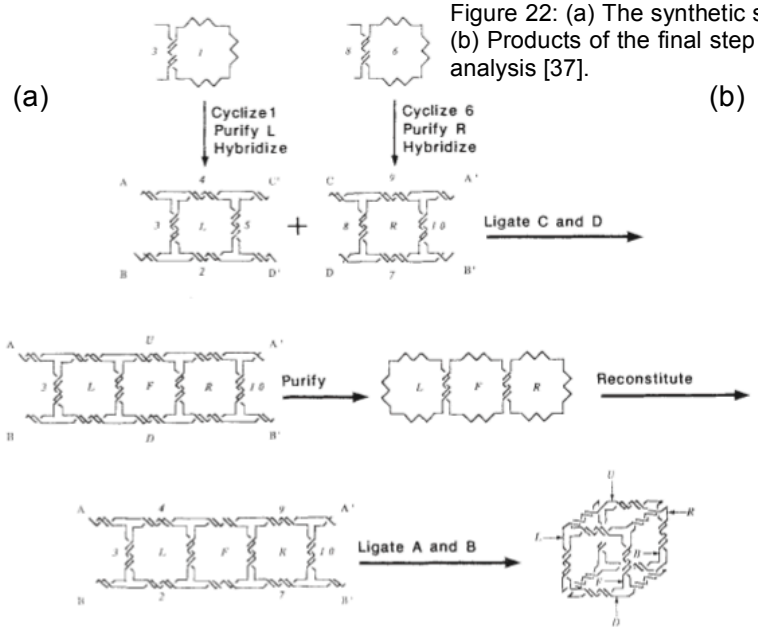
Figure 21: AFM image of 2D DNA scaffold. The unit cell is 20.5±0.2 and 21.1±0.5 nm, in good agreement with the expected dimensions. The features seen are fused helices, separated by two turns of double helix [36].



In 1991, Seeman's group also achieved a simple three-dimensional structure, with the connectivity of a cube. At the time, it was the first construction of a closed polyhedral object from DNA. Cyclizing, purifying, and ligating 80-bp circles of ssDNA can achieve this (see **Figure 22**a) [37]. Cyclizing is where you take linear DNA and ligating the strand to its other end, making a cyclic DNA molecule, although this definition does not seem to directly apply to the paper, from what we know. Again, the procedural details of their method are rather complex compared to our simplified understanding of biochemistry. But the publication gives information on the exact bp sequences, the enzymes, etc. used in the laboratory. Electrophoretic analysis gave proof that the DNA cubes were indeed made by their procedure (see **Figure 22**b).

Perhaps most amazingly, Seeman's group successfully engineered a three-dimensional scaffold based on a three-terminal unit in 2010. Seeman group refers to the structure made as a "tensegrity triangle" (see **Figure 23**). The tensegrity triangle occurs when ssDNA overlaps in alternating ways to make a triangular nano-structure held by tension. Each unit cell, or building

Figure 22: (a) The synthetic scheme used to synthesize the cube-like object; (b) Products of the final step of ligation, showing formation & electrophoretic analysis [37].



block, is made using 3 identical ssDNA segments and complementary strands. To make this structure (see **Figure 23**), one of the complementary strands needs to be circular, in the middle, and then three hook-like strands with identical base sequences on each corner of the triangle [38]. Once the structure self-assembles, this forces the DNA strands to stay within 78° of each other. The *GA* and *TC* sticky ends, on all three sides, symmetrically, ensure that any connectivity will give this pre-calculated unit cell.

Since designing the above structure constitutes the bulk of what makes this idea work, the procedure of this process is more straightforward. It would appear that the DNA synthesizer simply makes the strands of with these bp sequences. And then, annealing the right molar concentrations of each strand makes the appropriate crystal structure. Slow annealing made the DNA crystals: the temperature was incrementally lowered from 60°C to ~20°C at a rate of 0.2°C per hour [[38]]. Over the course of this process, which took a total of 7 days, the volume of the drop was reduced by 90%. After the cooling step, crystals appeared full-sized within a day. By the end of the process, Seeman's lab obtained well-ordered rhombohedral crystals with dimensions as large as 250 x 250 x 250 μm (see **Figure 23**) [38]. So far, this is the first example of converting the products of an in-solution process into a solid phase outcome.

How could such synthesis schemes allow us to integrate electrical functionality into these DNA scaffolds? Seeman's group has also demonstrated a quantum dot array based on a two-dimensional DNA scaffold, based on sticky ends [39]. Basically, imagine that after the self-assembly of a 2D DNA array, each block has a sticky end coming off of it. The sticky ends on throughout the array can either be assigned the same base pairs or different bp sequences. In this way, the bp sequences can act like molecular addresses. Seeman's group has shown that this principle works in the laboratory. They used quantum dots of 2 different sizes and functionalized them with ssDNA that had complementary bp sequences (see Figure 24) [[39]]. A TEM image shows the extent to which the laboratory efforts to "target" particles to specific locations (see Figure 24). So this experiment's achievement was encoding the self-assembly of

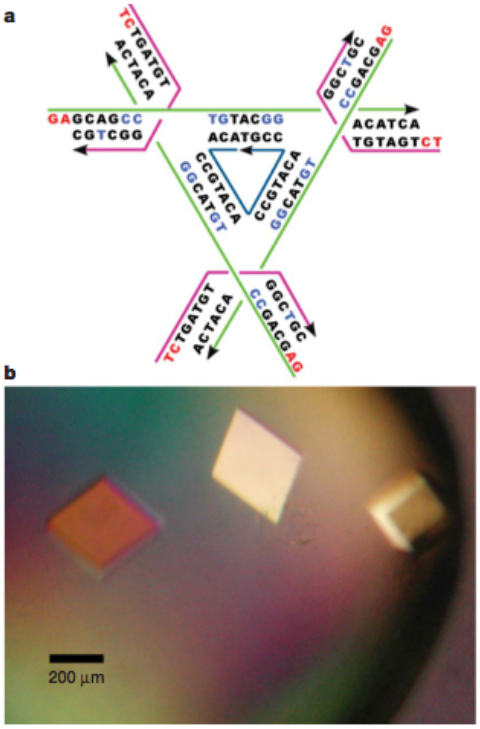


Figure 23: (a) Schematic of tensegrity triangle with bp-sequence; (b) An optical image of crystals of the tensegrity triangle. The rhombohedral shape of the crystals and the scale are visible [[38]].

different nano-elements by bp sequence. The irregularities in the array were products of the choice of bp for the bp sequences. Because of the bases chosen, non-specific interactions between the particle-bound bp sequence and the Au particle surface played an unforeseen role. These interactions interfered with proper hybridization to the scaffolding complements.

Overall, Seeman's group's work strongly implies that there is hope for endeavors of using DNA as a scaffolding material, for nanoelectronics. But it is possible that the complexity of their design procedures may limit the application of their products. According to Rothemund and others in the field, the work completed by his lab is, although amazing, not easily reproducible. And given what we know, these arrays and these methods do not suggest ways of integrating electrical functionality.

Zooming out, DNA nanotechnology has a lot to offer with respect to the first challenge of nanoelectronics (providing positional accuracy on the molecular level), but it has limitations too. Challenges include the high cost of custom DNA, initial high error rate of biological self-assembly (with respect to DNA Repair) [40], and finding a way to speed up the process of annealing or encourage bonding further. So there is room for improvement.

Our second challenge is controlling conductivity. Optimally, the electrical properties of DNA would be easy to measure and DNA *was as* conductive, or had similar conductivity, to silicon. But, as it is an organic material, it may not be a suitable metal OR semiconductor. Measuring these values are complicated, and deal mostly with this commonly unresolved transition between the in-solution phase and solid phase. So DNA may make a great scaffolding material, but as a molecular wire may be not so much: thus we cover this in Chapters 5 & 6.

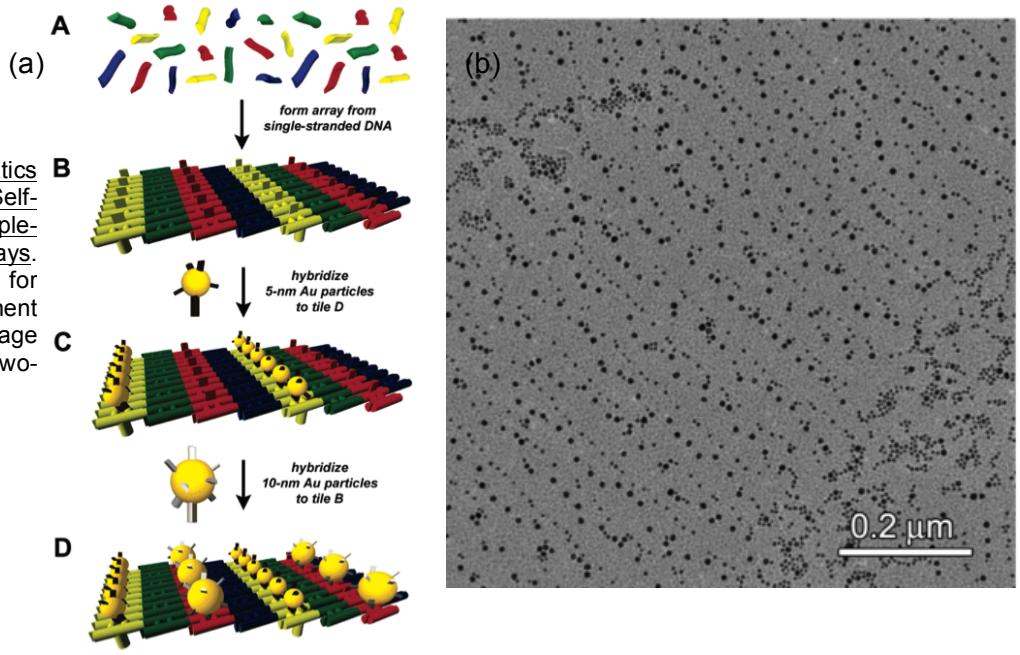


Figure 24: schematics and results for Self-Assembly of Multiple-Nanocomponent Arrays. (a) Assembly steps for the 2D nanocomponent arrays. (b) TEM image of the resulting two-particle array [39].

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Picture references:

- DNA sticky ends (http://www.mhhe.com/biosci/esp/2001_gbio/folder_structure/ge/m6/s1/index.htm)

CHAPTER V.

DNA Conductivity & Alternatives

So far in all the previous chapters, the electrical characteristics of DNA have had little to no contribution to our discussion. But, electrical properties of DNA *will* play a definitive role if we are suggesting them for nanoelectronic applications. The previous chapter assumed that DNA is, electrically speaking, a good insulator and electrically insignificant. The tools available for DNA in solution are unique to it, and make it a powerful molecule to our end-purpose. But our end-product will definitely not be in-solution, so the phase DNA is in must be fixed somehow. The question being, how do we resolve between the DNA characteristics we want and the ones we need to work with DNA in? The purpose of this chapter is to exhibit how involving biological self-assembly impacts our solution to the problem of maintaining control over conductivity at the nano-scale.

The first section of this chapter addresses the conductivity of DNA. DNA's conductivity has been up for debate for about 30 years as results depend heavily on the experimental setup. Interactions between the phosphate groups on the DNA backbone and ions in solution play a key role. These problematic interactions complicate electrical matters and the problem of converting DNA from solution-phase to solid-phase. Before trying to solve all these issues, we consider the situation that a working alternative exists. An appropriate alternative would have the benefits of DNA and the conductivity properties we are in search of. Alternatives explored include deoxyguanosine and peptide nucleic acid (PNA). But with both of these options, we lose some aspect of biological self-assembly that is beneficial to our greater purpose. Thus, a better alternative may be to work with DNA but to controllably alter its conductivity. That possibility will be explored in Chapter 6.

Because this chapter deals with the conductivity of an organic molecule, DNA, let us see how conduction translates into organic chemistry terms. You may have noticed how, in organic chemistry, a mechanism uses many double-headed arrows. Each arrow represents the nano-scale movement of electrons. Hence a nano-scale "current" would somehow involve some sort of chemical chain reaction favoring the *continuous* movement of electrons, ideally in a way that would reinforce itself.

When atoms of different electronegativities or certain functional groups are involved in carbon-based molecules, these non-carbon elements either add or withdraw electron density into a molecular system. Nitrogen in its natural, uncharged state makes a total of three bonds,

and has a lone pair of electrons. Oxygen naturally makes two bonds, and is also home to two lone pairs. When these atoms gain one extra bond (gaining more than one extra bond is extremely unlikely), they become cations, or positively charged atoms. When these atoms house an extra lone pair of electrons, they become negatively charged atoms, i.e. anions. Phosphorous typically makes three bonds like nitrogen, but because of the extra electrons in the *d*-orbital it can make up to five bonds. In the phosphate groups on DNA's backbone, for example, phosphorous makes five bonds with oxygen. In the backbone, oxygen is bonded to carbon because that is more favorable than phosphorous directly bonding to carbon. All of these oxygens on the phosphorous atom make the DNA backbone so negative.

We will talk more about how electron withdrawing groups and electron donating groups in the next section. When these groups are in the same molecule, they can induce a small dipole, which can induce a current, of sorts, when used properly. This comes in to play when we discuss how the bases—*A*, *G*, *T*, *C*—contribute to the "conductivity" of DNA. Let us see what type of charge transport dictates electrical conductivity in DNA.

A. Conductivity of DNA

When experimental evidence that DNA could behave like a metal AND semiconductor came forward, this aroused great interest in its possible use in nanoelectronics. The hope was that DNA and its supporting-role proteins could be used to build nanoelectronic circuits that self-assemble *and* are functional. Unfortunately, as much greater care was taken to eliminate extraneous effects, most investigators came to realize that DNA alone is, at best, a *weak conductor*. But it is a rather hard to resolve field because different experiments have predicted practically every type of electronic property.

To break it down, DNA's conductivity depends on base pair sequence, length, environmental conditions, and the nature of the contacts used for the measurement. First we address how each of these factors affects the conductivity of DNA. Then, we will cover what governs charge transport in DNA.

The Effect of Base Pair Sequence

Base pair sequences matter specifically because electron hopping between guanine residues seems to dominate DNA conductivity [41]. Using λ-DNA (the DNA of a virus that can only infect *E. coli*) one can begin to differentiate between the conductivity of DNA segments with near random base pair sequences, and those with high densities of *G* residues. For example, photochemistry can be used to create an electron-hole pair, which forms readily on guanine [42]. Inducing a current in the strand causes the hole to move, and electron movement,

conversely. There is a significantly higher ionization potential for holes residing on adenine, cytosine, or thymine bases compared to holes residing on guanine. The end-effect is that electrons moving along the DNA strand "hop" from guanine to guanine incoherently. Measuring DNA fragments cut at different G sites can help measure the probabilities, and the relative rates of hole transfer along the strand [[42]]. And the rate of hole transfer implies the movement of electrons, as both will move in opposite directions under an applied field. The experimental inferences hold that electrons hop incoherently between *G*'s along the strand, making a random walk. But when moving from one guanine to the next, the electron does not stop mid-journey so coherent super-exchange can describe the transfer between two guanines [[42]]. But just one *G*-*T* or *C*-*A* bp mismatch in a 15-bp strand of dsDNA increases the resistance by about 300 times compared to a correctly-matched bases [43]. But in general, these guidelines explain why guanine plays such an important role in future investigations of DNA's conductivity.

Organic chemistry can explain why guanine is more conductive than all the other nucleo-bases. Both adenine and guanine have more extended pi-conjugated systems compared to their monocyclic, pyrimidine counterparts. The bicyclic rings of a purine can stabilize greater electron densities, compared to the pyrimidine rings, by resonance (see **Figure 25**). In organic chemistry, it is common to represent the "rest of a molecule" using "R", so let us use this convention to talk about the functional groups on adenine and guanine, and compare the two. R—NH_2 and R_2—NH are both electron-donating groups, and they donate their lone pair to make a double bond with the associated carbon and take on a positive charge. R=O is an electron-withdrawing group, which works by withdrawing a bond so that it has three lone pairs and only one bond, taking on a negative charge. Notice that adenine only contains electron-donating groups, whereas guanine has both electron-donating and electron-withdrawing groups. How adenine has all electron-donating groups explains why the energy of a hole residing on adenine is substantially higher than the energy of a hole residing on guanine.

But notice that experiments using λ-DNA will not be able to single out other phenomenon. Experimentally, making DNA that is made up of only one repeating base pair is one way of taking base pair sequence out of the discussion. One such type of dsDNA could be only guanine, all down one side and only cytosine down the other strand: this is called poly(*dG*)-poly(*dC*)

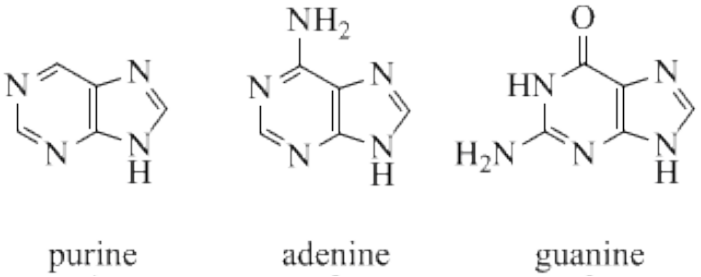

Figure 25: The chemical structure of the DNA bases that are purines. They are called "purines" because they have the same basic structure as a molecule called purine.

DNA. The converse of this would be dsDNA with only adenines down one side and only thymine down the other: this would be poly(*dA*)-poly(*dT*) DNA. Another variation of a "control group" sequence would be *GCGCGCGCGC* etc., i.e. poly(*dG*-*dC*) DNA. So experimentation to gauge the effect of other factors usually will be using one or more of these later DNA motifs, base-pair sequence wise.

Effect of Length, Solvation Effects, and Contacts

The length of DNA matters for short-range conduction, but after a sufficient length, the size does not matter any longer. Coherent super-exchange explains metallic behavior in DNA strands less than 8-bp long. For poly(*dG*-*dC*) DNA, the conductance (in solution) is inversely proportional to the length, when the DNA is more than 8 bp long.[44]. When inserting *A*-*T* nucleotides into *G*-*C*-rich areas, the conductance decreases exponentially with the length of the poly(*dA*)-poly(*dT*) segment (in bp) with a decay constant of 4.3/nm [44]. In terms of base pairs, the above finding correlates to a decay constant of about 1.4/bp. These findings confirm the dominance of base-sequence dependence of DNA conduction over backbone-dependence.

DNA exhibits metallic or semiconducting behavior on the short-range [45]. A definitive descriptor for long-range electronic activity is hard to pinpoint, though. The electron transfer mechanisms that come into play differ in their dependence to sequence. Quantum tunneling effect is distant-dependent, as the rate of charge transport decreases exponentially with increasing distance between guanines. The incoherent hopping of electrons between G bases gives a weak, algebraic decay of transfer rate with length [42]. On the contrary, in incoherent hopping, the distance dependence is expected to be less prominent. Coherent super-exchange explains short-range electron transfer, but incoherent hopping describes long-range electron transfer [42].

The backbone of DNA is negatively charged, impacting both conductivity and the ability to convert solution-phase DNA into solid-phase DNA. The negatively charged phosphate groups along the backbone of DNA inhibit the flow of electrons [46]. Counter ions, positively charged species, can bind to the phosphate ions and render them electrically neutral [47]. But since counter ions are hard to uniformly apply to the backbone, conductivity depends strongly on these structural changes induced by the solution's ionic content [47]. So, experimentally, the

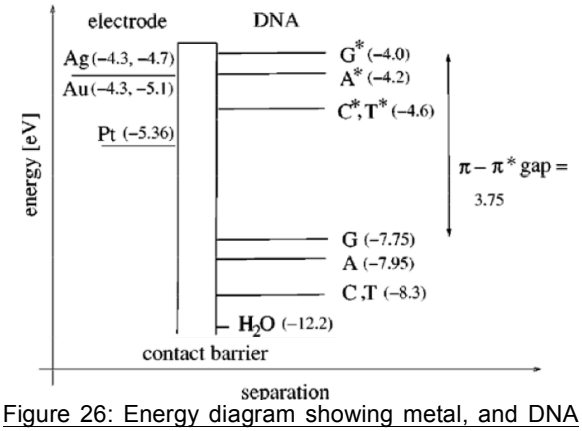

Figure 26: Energy diagram showing metal, and DNA work function [48]. Adding the gap size to the single-base ionization energies gives the excited-state energies of the bases.

presence or lack of counter ions might contribute to the appearance of DNA being insulative, semiconducting, or metallic. This makes the choice of solution, or "environmental conditions," highly important. The choice of counter ions also impacts phase conversion, so methods of preparation matter also, because it effects whether salts are left on the backbone.

In the experiment, the nature of contacts, or how a DNA molecule or "rope" is attached to the two electrodes, presents a key experimental challenge. Reproducible electronic coupling, between DNA and the probing electrodes, is necessary to measure conductance reliably. AFM, STM, and low-energy electron point source technology help fabricate nano-electrodes [48]. Semiconducting behavior in DNA arises when the bp in the sequence in short DNA molecules (<100 bp) have a large HOMO-LUMO gap (>4 eV) with the metal work functions sitting inside the gap (see **Figure 26**). Sticky ends are then typically used to attach the DNA to the electrodes. Many technical and reproducibility obstacles are met, with respect to all of the aforementioned things DNA conductivity depends on. Usually, the use of thiol groups (R—SH) compromises the reproducibility of the experiments. One group reported metallic for and semiconducting behavior if there is a mismatch, with carbon nanotubes as electrodes [43]. Their use of robust amine linkages avoids the thiol groups, showing hope for more reproducible methods. Reproducible experiments varying the type of contact made without changing the DNA molecules would be helpful. But such experiments have not been conducted, so the effect of contact geometry or style is hard to determine. Since no one way of forming contacts is ubiquitously accepted as the most suitable and stable contact structure, there is no standard way of forming contacts either.

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In experimental results for conductivity, all of these effects seem to manifest themselves in confusing and hard to analyze ways. In general, experimentalists can measure conductivity in one of two ways: either using indirect electrochemical or direct transport measurements. Using either approach, a variety of sources have reported insulative [49],[50], semiconducting [51], metallic [52], and even superconductive [53] behavior. Clearly, only one of these adjectives should describe DNA's electrical behavior. The insulative behavior seems to come from the use of 1.8- to 16-μm λ-DNA, in two different environmental conditions [49],[50]. The use of a 10.4-μm-long, 30-bp-long, poly(*dG*)-poly(*dC*) DNA measured on 8-nm electrodes shows semiconducting behavior, with a large band gap [51]. DNA appears to behave like a good linear conductor in the form of micrometer long DNA ropes and DNA-based thin films [51]. Particularly, 600-nm λ-DNA "ropes" (2-4 dsDNA molecules stuck to each other in a larger strand) were found to have a resistance of 2.5 MΩ. The superconducting behavior has basically been disproved: it was a solution-dependent kind of long-range coherent transport that was being observed [42].

The experimental results from different groups vary widely so there is a lack of a consistent picture.

Another apparent problem is that DNA has a hard-to-remove water layer in the major & minor grooves under practically any conditions. There is experimental evidence that conductivity of DNA arises from water molecules, not the electrons passing through on the bases [54]. So working with DNA in solution phase comes with its own problems. Some of the aforementioned papers use an enzyme that makes a blunt-end dsDNA break to ensure that it is indeed the DNA conducting [[51]]. By cutting the DNA, this also discontinues the line of water molecules along the grooves of DNA. So making the dsDNA break still does not conclusively disprove the conjecture that the water molecules in the grooves are conducting. So the enzyme-cutting approach appears to be an insufficient method of ensuring that DNA is the thing conducting electrons.

We would be lucky if mathematical models for charge transport matched up closely with any one of the aforementioned experimental results. That may have indicated what theory could explain, at least; but unfortunately we have less to work with. DNA is a complex molecule, and representing its aperiodic chemical structure presents a major problem for charge transport investigations. The way bases stacking works makes direct self-consistent field (SCF) calculations about DNA's electronic structure practically impossible [55]. Hence approximate methods need to be employed. As far as mechanisms go, incoherent hopping transport & coherent transport both are models used to explain charge transport in DNA. Coherent transport happens through pi-pi interactions between stacked base pairs. These interactions lead to extended states with a reduced DNA energy gap that could explain metallic DNA [55]. Despite numerous intensive investigations, the actual mechanism for charge transfer in DNA remains controversial. So even an understanding of the underlying mechanism does not provide a complete explanation of DNA's electronic structure.

Where the field is right now puts a huge responsibility on experimentalists, but there may be hope in clarifying conduction in DNA. Reviews summarizing and explaining all the different types of electronic behavior of DNA exist [48], but none have received this research community's undivided support. Nonetheless, there is hope because scanning tunneling spectroscopy has helped disclose the energy spectrum of poly(dG)-poly(dC) DNA molecules [56]. In a sense, this "decoded" the electronic density of states of DNA. For this process, the dsDNA was deposited on gold, and measurement took place at cryogenic temperatures. Ag^{2+} ions functioned as counter ions, and caused the average gap widths to reduce by one-fourth. By means of *ab initio* density functional theory calculations, the origin of the peaks could be

attributed to the nucleobases, the backbone, and the counter ions. More work that concretely relates electronic structure to the variety of factors playing a role will definitely help elucidate what drives electron transfer through DNA, someday.

Beyond what we have already discussed, the effect of temperature on DNA's conductivity is more or less outside the scope of this paper.

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To summarize the preceding, DNA exhibits a wide variety of electrical behavior, depending on base pair sequence, length, and solvents used. Experiments showing metallic behavior in DNA used DNA bundles, ropes, or supercoiled DNA with solvent molecules trapped in between, possibly [48]. DNA exhibiting wide-band gap semiconducting behavior is usually short (8-100 bp) and has a homogeneous bp sequence [48]. DNA acts like an insulator when the DNA has either a nonperiodic bp sequence (such as λ-DNA) or the chemical treatment of DNA has completely destroyed the DNA's helical structure [48]. The superconductive behavior in DNA is debatable, and mostly unrelated to our purposes. These explanations may partially summarize the causes and effects in DNA conductivity. But the effects of counter ions and the nature of the contact still make it difficult to draw firm conclusions about how to make DNA behave a certain way.

Let us apply these concepts to what we know so far. Initially, we were hoping that DNA would behave like a perfect insulator when using Seeman's approach to DNA scaffolding. But now we know we cannot assume that. According to the discussions in this section, the conductivity of DNA is more like small nm-sized segments of λ-DNA, and probably semiconducting. Charge transport would be governed by incoherent hopping on the short-range, in the tensegrity triangle scaffold for example.

So although DNA was a good starting place, it comes up short as a molecular wire, because its low and/or irregular conductivity. DNA was able to illustrate everything that could be possible in the way of self-assembly, using enzymes, self-assembly of nanoscaffolds, and more. But it does not seem like the conductivity of DNA itself can be easily and consistently controlled. Thus, one must find ways to precisely control the products of preparation as well as develop reproducible methods of measuring DNA's conductivity. So many problems come with choosing to use DNA as a nanoelectronic material, even as a scaffold. Hence, we should probably consider alternatives to DNA and entertain the possibility that DNA is not the right material for our use.

Let us see if alternatives exist whose electrical conductivity properties are easier to control or model, AND can self-assemble.

B. Deoxyguanosine

If guanine is the most conductive of the bases in DNA, why not make version of DNA that uses only guanine, and no cytosine? Compared to DNA, guanine *can* be modeled mathematically, and this is useful because bp sequence dominates conduction anyway. A research group in Italy, collaborating with other European counterparts, tried to take this concept from mathematical model to experimental reality. Di Felice's group has done a significant amount of work on analyzing the conductivity of DNA [41], [43], [56] so let us see how they use that information to their advantage.

Typically, base pairs couple using hydrogen bonds, where the donors and acceptors govern the direction of dipoles set up in the molecules. In the classic, Watson-Crick base pairing, *G* pairs with *C* and *A* pairs with *T*, which are all planar molecules. The R_2-NH and R-NH_2 groups act like hydrogen donors and the sp^2 hybridized oxygen and nitrogen atoms with lone pairs act like the hydrogen acceptors (see **Figure 28**). But it is possible to form other base pairings via hydrogen bonding too. Particularly, this research group depends on Hoogsteen pairing to produce base stacks of guanine (see **Figure 28**b). The G-4 complex, or G-quartet, refers to when 4 guanine molecules form hydrogen bonds to each other (see **Figure 28**b).

Figure 28: Watson-Crick base pairs participate in hydrogen bonding.

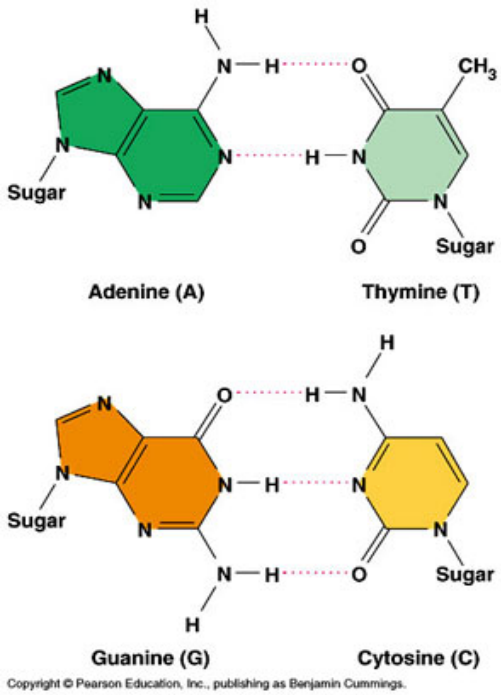
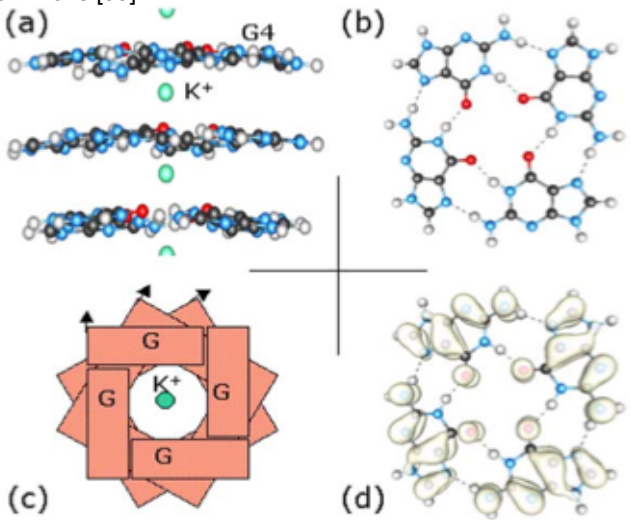


Figure 28: Alternative H-bonding involving only guanine. (a) Side view of G-quadruplex stacks in DNA, without backbone; (b) Top view of geometry of isolated G-quartet, showing H-bonding; (c) Top view of how each stack rotates through DNA (each rectangle represents a guanine molecule); (d) HOMO state of the isolated G-quartet. Colors refers to different chemical species. White stands for hydrogen, blue is nitrogen, red is oxygen, grey is carbon, and green stands for K ions [59].



Because of the unique order of hydrogen donors and acceptors on the guanine molecule, the chemical self-assembly of guanine into various structures is in fact favorable.

Figure 30: Chemical structure of lipophilic guanosine. 3',5'-di-*O*-decanoyl-29-deoxyguanosine, also referred to as lipophilic guanosine, self-assembles in the presence of alkaline picrates.[57]

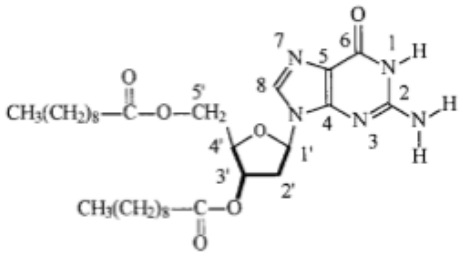
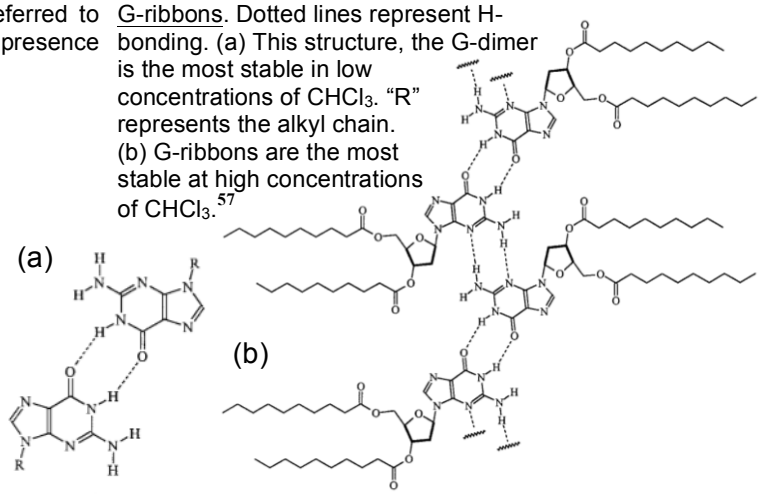


Figure 30: H-bonding of a G-dimer and G-ribbons. Dotted lines represent H-bonding. (a) This structure, the G-dimer is the most stable in low concentrations of $CHCl_3$. "R" represents the alkyl chain. (b) G-ribbons are the most stable at high concentrations of $CHCl_3$.[57]



Hydrocarbon solvents like chloroform facilitate the self-assembly of various homo-guanine structures from lipophilic guanosines [57],[58]. Lipophilic guanosine looks like a nucleotide with guanine on it, but it has alkyl (hydrocarbon) chains on it instead of the phosphate groups (see **Figure 30**). Typically, DNA nucleotides with the phosphate groups do not self-assemble into long-range supramolecular structures without the aid of enzymes. Self-assembly is more favorable in this molecule because alkyl chains replace the mutually-repulsive phosphate groups on the nucleotides. These hydrocarbon chains can bind to each other via nonpolar Van der Waals interactions, which increase in strength with longer chains. At low or high concentrations of lipophilic guanosine in $CHCl_3$, lipophilic guanosine can self-assemble into dimeric (see **Figure 30**a) or polymeric forms (i.e. G-ribbons, see **Figure 30**b), respectively. In the presence of K+ ions, G-4 monomers (see **Figure 28**b) are converted into octamers (see **Figure 31**), based on G-quartets. Increasing the concentration of K+ ions further transforms the octamers to polymeric columnar species, making the G-quadruplex molecular wire. Thus, G-dimers, G-ribbons, or G-quadruplex can be formed depending on solvent concentrations and the presence (or absence) of specific cations.

Out of all of the homo-guanine structures considered, G-quadruplex or G-4 wires are the most interesting because they are similar to DNA in structure. The core of Di Felice's group's work is mathematically modeling the electrical conductivity of DNA and G-4 wires. In G-4 wires, the structure and geometry of the stacking affects the electronics of the wire. The dispersion of the highest valence band and the lowest conduction band is considered negligible in the x-y plane in which the guanine lies. But something different happens to the electron dispersion in the z-direction perpendicular to the plane containing the G-4 complexes. In the z-direction, the dispersion shows high sensitivity towards how the bases are stacked. If stacked correctly, the π-π interactions between G-4 layers provide a pathway for rapid one-dimensional charge transport [59]. At first, Di Felice's group suggested G-4 wires as DNA-based molecular wire with improved conductivity, based on their ability to optimize stacking for improved π-π interactions.

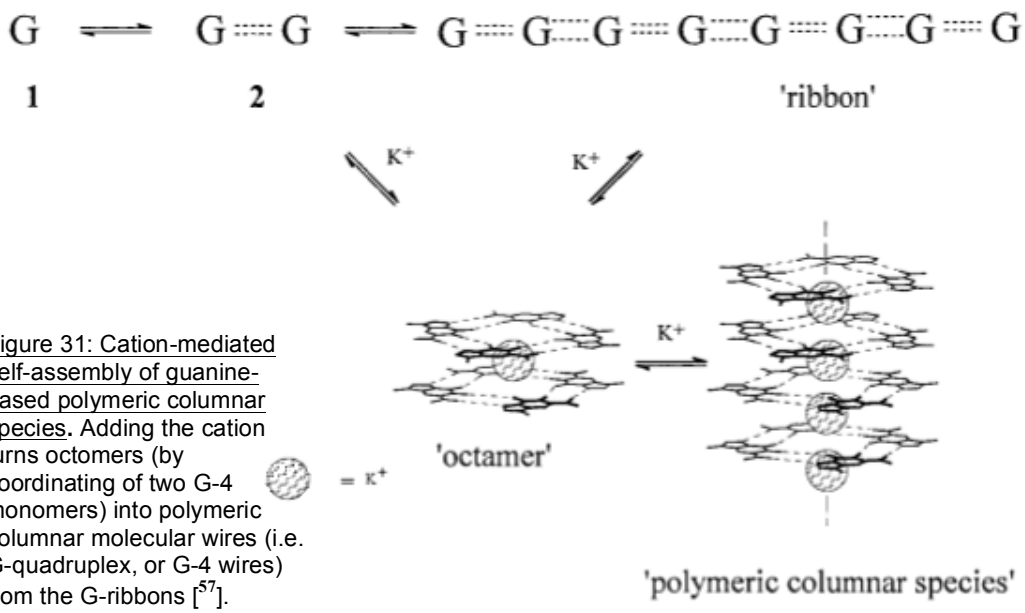


Figure 31: Cation-mediated self-assembly of guanine-based polymeric columnar species. Adding the cation turns octomers (by coordinating of two G-4 monomers) into polymeric columnar molecular wires (i.e. G-quadruplex, or G-4 wires) from the G-ribbons [57].

But modeling the charge transport through G-4 wires shows that the conductivity of this species is not much different from when DNA behaves like a semiconductor. So the Di Felice group's treated the electronic properties of all the model solids as inorganic wide-band gap semiconductors. Modeling showed that super-positioning of the π orbitals between stacks was not enough to induce band dispersion along the wire axis [60]. Part of their group effort was dedicated to trying to empirically reproduce the graphs made using theory. But many technical and reproducibility obstacles have prevented the Di Felice group from getting usable direct measurement of conductivity.

The additional K+ ions do not significantly improve the conductivity of the molecular wires [61]. But the conduction properties of the nanowires could probably be tuned by using different metal ions to stabilize the stack. Although Di Felice's group has mentioned this possibility in their later works on G-4 wires, they apparently did not follow up on this investigation. The intercalation of metal ions in a base-pair stack is a new idea: and we have not really considered this option for DNA. Perhaps, even DNA's conductivity could benefit from this.

There are negative repercussions to changing the backbone of the nucleotide and to using only guanine. Without the ability to play with the base pair sequence, we lose our ability to make location specific adjustments or addresses. Using sticky ends on bases with less bulky alkyl groups (with shorter or smaller R groups) may work to make up for this. Sticky ends may allow us to incorporate it in the DNA scaffolds mentioned in previous chapter, but there is no evidence of this working in literature. Also, without an enzymatic toolkit that works on deoxyguanosine derivatives, it would be hard to make into scaffolds with lipophilic guanosines. Perhaps, moreover, the Di Felice group never even ventured toward building laboratory-scale G-4 wire-based devices.

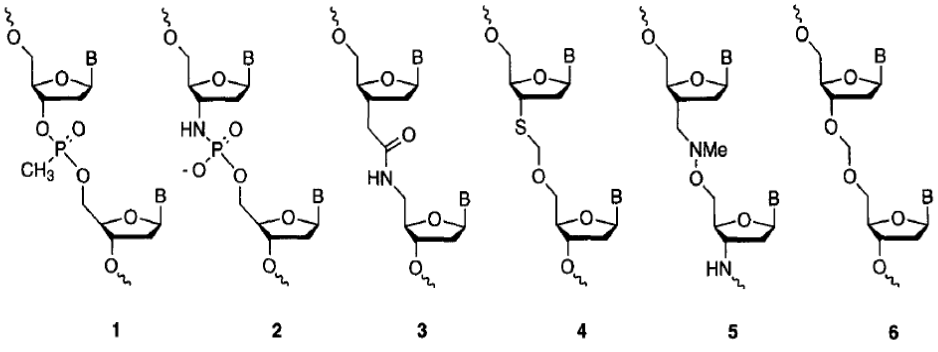
So although G-4 wires can be self-assembled at the most basic level, the higher types of self-assembly are not available to deoxyguanosine. So let us see if there is something more analogous to DNA that could give us more control over conductivity while giving us a fuller range of the self-assembly capabilities.

C. Oligonucleotides

Outside DNA-based research, there exists an entire world of molecules that use the same bases (with Watson-Crick pairing), but have a completely different backbone. Ribonucleic acid (RNA) is a naturally occurring molecule that is closely associated to DNA's function in the body. Unlike DNA, RNA nucleotide *has* the –OH group on the 2' carbon, (for a better explanation, see **Figure 5**). Some of the methods of scaffolding and molecularly manipulating DNA may also apply to RNA because it, too, has convenient modifying enzymes. All of the DNA-binding and RNA-binding proteins that occur naturally & biologically "recognize" features of the DNA and RNA backbone. The recognition is based on the Hydrogen-bonding formed with the backbone or base-pair stack, accessible by the major groove. But, electronically, that problematic phosphate group ($-PO_4$) is still there on RNA's backbone. So we turn to other kinds of nucleotides, besides DNA and RNA, which also use Watson-Crick pairing.

Oligonucleotide synthesis is the area of molecular biology research dedicated to the synthetic chemistry of short-chains of nucleic acid polymers. In this paper, we are particularly interested in oligonucleotides with either modified or completely re-engineered backbones. Because these molecules can be prepared completely synthetically, their backbones can be engineered to have almost any property. Note that, oligonucleotide synthesis can barely ever exceed 200 bp. But nanoelectronics could benefit from a chemically neutral backbone that does not alter the electronic function of our wire, or even a species that conducts well.

Figure 32: A variety of synthetic oligonucleotides **(1) methyl phosphonate nucleotides**, where a methyl group replaces an oxygen atom in PO_4); **(2) N3'→P5' phosphoramidite nucleotides**, where a amide or –NH group replaces the oxygen on the 3' carbon; **(3) Amide-linked nucleotides**, where the backbone consists of a 3'–CH_2-NH-CO-CH_2-5' chain; **(4) 3'-Thioformacetal-linked nucleotides**, where the backbone consists of a 3'-S-CH_2-O-CH_2-5' chain; **(5) methylhydroxylamine-linked nucleotide**, where the backbone consists of a 3'-CH_2-NCH_3-O-CH_2-5' chain; and **(6) Formacetal-linked nucleotides**, where the backbone has a 3'-O-CH_2-O-CH_2-5' chain [62].



Perhaps one of the oligonucleotides (i.e. oligos) that have already been designed may fit our needs (see **Figure 32**) [62]. Most of these oligonucleotides are designed to resist certain types of enzymatic activity in physiological conditions. That enzymatic resistance is achieved by making changes in the inter-nucleotide bonds. Let us incrementally elaborate on how different inter-nucleotide changes affect the natural charged state of the backbone, by discussing the molecules in **Figure 32**:

(1) Replacing the negatively charged phosphodiester linkage with the nonionic methylphosphanate (see **Figure 32**, oligonucleotide #1) makes an uncharged DNA analogue. Without the negative charge repulsion of the backbone in this analogue, it denatures at a higher temperature than regular DNA. The single-stranded version of this oligonucleotide can bind to DNA with a high degree of specificity, bp-wise, to make a short triplex along the double-stranded form. Changing the phosphate group like this also makes this analogue resistant to cleavage by nucleases.

(2) Using nitrogen on the backbone (see **Figure 32**, oligonucleotide #2) is another way of making the backbone more neutral in physiological conditions. Here, a higher denaturation temperature is also observed in literature. Again, Watson-Crick pairing helps single-stranded analogue bind very specifically to DNA. Replacing the 3' oxygen with nitrogen changes the hybridization state of the backbone and changes the whole helix's structure. Effectively, the major groove deepens and the minor groove becomes very shallow. The altered backbone means oligonucleotide #2 cannot be cleaved by nucleases either. The change in the helix structure may also make it harder for other DNA-binding proteins to form the appropriate bonds with the backbone or bases.

Oligonucleotides #3 to #6 involve changing the entire backbone of DNA. This means the true length of the replacing chain must match that of DNA to retain the DNA-binding ability.

(3) Oligonucleotides using amides as a new type of backbone (see **Figure 32**, oligonucleotide #3) still show good affinity for complementary DNA strands. These molecules exhibit an increased denaturation temperature, and are resistant to hydrolysis and 3'-exonucleases. The hydrogen on the nitrogen, or the imino proton, could even be replaced by functional groups, without compromising any of the features.

(4) 3'-thioformacetal-linked nucleotides (see **Figure 32**, oligonucleotide #4) can bind to dsDNA, form triplex because backbone length is right. The relatively neutral backbone also results in an increase in denaturation temperature. These backbones are completely resistant to restriction enzymes.

(5) But oligonucleotides do not always have all three of these features. For example, methylhydroxylamine-linked nucleotides (see **Figure 32**, oligonucleotide #5) have higher denaturation temperatures too, and can be resistant to nucleases. But they do not have high sequence specificity with DNA.

(6) Formacetal-linked nucleotides (see **Figure 32**, oligonucleotide #6) are another counterexample to those aforementioned. This oligo exhibits a decrease in denaturation temperature and binds poorly to dsDNA (because backbone length falls short). But still, this oligo is resistant towards nucleases.

Because these oligonucleotides were designed for biochemical experimentation, their electrical properties are not defined in literature. In the absence of backbone conductivity, incoherent hopping between the bases will dominate charge transfer. But what might one expect from the above backbones? Conductive polymers typically repeat molecules with conjugated pi-systems, aromatic rings, and the selective use of electron-donating and electron-accepting groups. To get electron flow going, the electron-donating or electron-withdrawing molecules are used to "dope" the polymer. At the injection or removal of a single lone pair, the electrons would cascade through the alternating double and single bonds of such a system. The careful use of atoms with different electronegativities can also supply more lone pairs. For example, nitrogen has one lone pair and oxygen has two lone pairs. But nothing in **Figure 32** seems to have a continuous pi-system along the backbone. And the discussion of what defines an electron donor and electron acceptor gets complicated fast: we will discuss this in Chapter 6. So let us focus on an oligonucleotide with electrically neutral backbones.

D. PNA

Perhaps staying away from an ionized backbone would suffice. One example of an electrically neutral DNA analogue is peptide nucleic acid (PNA), whose backbone involves nitrogen atoms and carbon-oxygen double bonds. Because PNA's backbone is similar to that of proteins (refer to Chapter 2, Section C for review), peptide synthesis techniques directly apply. We are interested in this particular oligonucleotide because it's charge-neutrality, it's apparent biochemical stability, and it's similar periodicity to DNA.

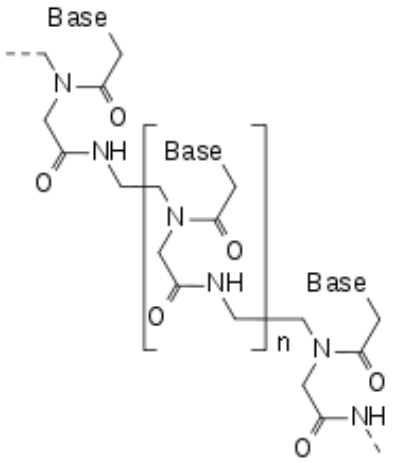


Figure 33: Chemical structure of PNA. The bracketed portion represents the repeating unit.

PNA consists of repeating N-(2-aminoethyl)-glycine units linked by peptide bonds (see **Figure 33**) [62]. The chemical structure of PNA is similar to oligonucleotide #3 from **Figure 32** in some ways, except a methylene carbonyl bond attaches the base to the backbone. There appear to be no studies on its mechanical properties, but because of its promise in medicinal chemistry, its biochemical properties are well understood.

PNA has many chemical possibilities, as it is stable over a wide range of pH. The liquid-to-solid phase transition maybe easier since PNA's backbone shows insensitivity to ionic solvents in solution [63]. But, note that the affinity of water molecules to the base-pair stack, accessible by the major groove, is still a matter of debate. Denaturation temperatures indicate that antiparallel PNA-DNA duplexes (where one strand is PNA, the other, DNA) are more stable than parallel duplexes [64]. Antiparallel implies that the N-terminus faces the 3' end and the C-terminus faces the 5' end of DNA. PNA-PNA duplexes have higher denaturation temperatures than dsDNA, and shows high sequence-specificity. Because PNA is achiral, the PNA-PNA duplexes that result from hybridization are a mixture of right-handed and left-handed helices.

Because PNA basically has peptide bonds throughout its backbone, well-established solid phase peptide synthesis techniques define its chemical synthesis [[62]]. Like peptide synthesis, PNA synthesis requires the use of "protecting groups". These are chemical additions to our actual molecule of interest that protect the bases or the places where we do not want the reaction to take place. Proteins do not mediate the actual synthesis at all: it involves cycling through 3 steps, for the addition of each monomer. First capping is necessary: this involves the application of protection groups. Then, a deprotecting step frees up the location of the desired addition (by removing only the relevant protecting group). The coupling reaction refers to when the next monomer is actually appended onto the PNA strand being made. PNA is achiral so

creating stereoisomers is not a problem, as it can be in the chemical synthesis of such molecules. The chemical synthesis offers full control over base pair sequence in PNA strands. This is a high purity and high yield chemical process. Making PNA monomers from scratch is hard [62] so buying pre-made monomers turns out to be expensive: the average price of 1 g of PNA monomer is around $500. And a type of chemistry, called 'click chemistry', may be able to extend the 200-bp limit of oligonucleotide synthesis for PNA.

The foreign nature of PNA's backbone makes it immune to practically every typical form of nucleic acid manipulation biologically available. This means either nucleases or proteases cannot recognize PNA easily, making them resistant to enzyme degradation. Using a strand of PNA that is complementary to a dsDNA can interrupt replication by making a triplex with the base pairs in the dsDNA. So all the forms of DNA-type self-assembly that involved enzymes is moot for PNA. This implies that PNA scaffolds would only be possible through chemical methods, not by enzymatic methods analogous to those used by Seeman's Group. But functionalizing PNA ends might make it available to some forms of self-assembly.

Either the addition of amino acids, sticky ends, or biotin and streptavidin, as a pair, could be used to functionalize PNA strands. The carbons between the carbonyl (C=O) and –NH group on the backbone is a potential location from incorporating amino acids. Adding amino acids to PNA's backbone could functionalize it. This type of functionalization could be used to fine tune hydrophilic or hydrophobicity, and even place a positive charge on the backbone, if desired. To incorporate PNA into DNA scaffolds, using sticky-ends might be applicable. Another way to functionalize PNA is using biotin, a water-soluble molecule (Vitamin H), and streptavidin, a cup-shaped protein used with biotin to bring together biomolecules [[63]]. Biotin and streptavidin have an incredibly high affinity for each other, one of the strongest non-covalent bonds known to nature. The dissociation constant, effectively the concentration of a ligand needed for a protein to recognize it, is $K_d = 10^{-14}$ mol/L for streptavidin and biotin. Notice that only a very small concentration of biotin is needed for streptavidin to "recognize" it: this implies how strong their bonding is. Streptavidin's affinity to biotin does not change with pH, temperature, organic solvents, denaturants, and many other chemical parameters.

The electrical characterization of PNA has not been as hotly debated as that of DNA. Using what we know, if the backbone is electrically neutral, we may guess that the base-pair sequence governs electron transport and its mechanism. And these notions have been confirmed in self-assembled monolayers of PNA: the hopping mechanism between the guanine residues predominates, resulting in insulative behavior [65]. By itself, PNA, coordinated with CNTs, can exhibit diode-like behavior, which is promising [66]. But the paper exhibits a singular

laboratory-scale demonstration of the device, without accounting for a way to make a mass array of such devices.

The chemical and biochemical limitations for PNA are defined by the extent of click chemistry. Click chemistry describes chemistry that is tailored to generate substances quickly and reliably, by joining small units together. It does not describe a particular reaction, and simply draws inspiration from nature in how it, too, can generate substances by joining small modular units. For the case of PNA, click chemistry can do the job of ligase, for example, but not much more than that. Such chemical techniques are still not as grandly efficient and parallel as the proteins that act on DNA strands. Basically, to make PNA as useful as DNA, a whole new field would have be dedicated to forming a family of special proteins that could efficiently modify PNA. Since DNA and its enzymatic toolkit already exist, this is like having to reinvent the wheel. These issues with PNA represent the variety of problems we would face with the use of any synthetic oligonucleotide.

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For both deoxuguanosine and PNA, we see the end effects of not having DNA's backbone. Without the same backbone as DNA, many DNA-binding proteins will not bind to the G-quadruplex strands nor act on them. We would have to resort to other forms of chemistry, or devise and invent new ones to ligate, cleave, unzip, zip, etc. Adding complexity may be harder without enzymatic toolkit.

So, in the next chapter, let us return to DNA, whose enzymatic toolkit is unmatchable, and see how to make an electrical device out of it.

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Picture references:

- Chemical structure of purine, adenine, and guanine (http://en.wikibooks.org/wiki/An_Introduction_to_Molecular_Biology/DNA_the_unit_of_life)
- Watson-Crick base pairing, with hydrogen bonding (http://weloveteaching.com/2011/organics/base-pairs.jpg)
- Chemical structure of PNA (http://en.wikipedia.org/wiki/Peptide_nucleic_acid)

CHAPTER VI.

Returning to DNA

It turns out that the very phosphate-filled backbone, which gave us trouble with electrical characterization and phase transition, is necessary to use the enzymatic toolkit. Some applications of DNA miss out on this biochemical opportunity when they use DNA in an electrical context. They return to DNA simply to make more laboratory-scale devices that do not use enough biological self-assembly.

Here are two examples of DNA-based, laboratory-scale, proof-of-concept transistors have been around for a while. For example, a Korean research group demonstrated the first single-DNA field-effect transistor in 2001 (see **Figure 34**) [67]. An Israeli group, who demonstrated a DNA-based template for CNT transistors, exhibited a second example of a DNA-FET [68]. By using biotin and streptavidin, the fabrication scheme is partially given to adding complexity, and we will discuss this more later. But because the scheme resorts to the use of presorted semiconducting CNTs, the overall experiment does not make full use of biological self-assembly. These experiments focused on making functional single FETs, which is useful for fundamental studies. But the fabrication techniques employed for these "hero" devices are still not amenable to circuit production, as per our standards: they are "anti-complexity", for our intents and purposes.

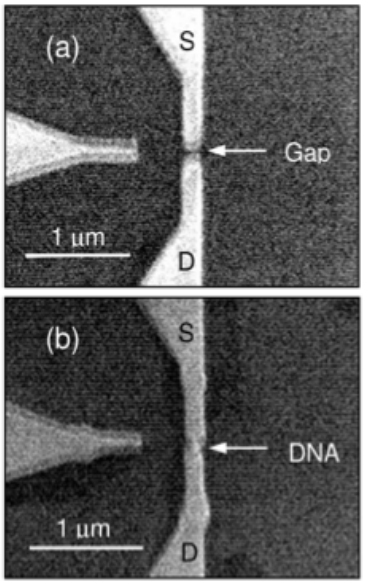


Figure 34: SEM images of a DNA FET. (a) the source and drain Au/Ti nano electrodes with a 20-nm gap. Three electrodes are shown but only the S and D electrodes were used. (b) SEM image of the poly(*dG*)-poly(*dC*) DNA molecule trapped between two electrodes [67].

This chapter is about how biological self-assembly can give us a way to place nano-elements with positional accuracy *and* control electrical conductivity *with DNA.* What we need is a way to make a next-generation transistor that uses the concepts we know to facilitate the large-scale addition of complexity. The same group at Technion mentioned earlier found a way to integrate biology and electronics in this very important way. We know that the purpose of returning to DNA is so that we can make use of its enzymatic toolkit, and its proteins. And their use of a protein called RecA, combined with other self-assembly techniques, may offer a plausible way to add complexity on this scale. But the metallization scheme used by the group at Technion renders the DNA backbone inaccessible to

proteins we would want to use. An alternative form of DNA that may help regain accessibility to the backbone and still improve conductivity is M-DNA. In M-DNA, metal ions coordinate with the base-pair stack via hydrogen bonding, but we will cover more on M-DNA later.

Using all of these concepts in conjunction, we propose a scheme of building an array of devices. In the second section, we integrate this idea with Seeman's 3D DNA scaffold and show how making an array of devices may be possible. But for the successful integration of these schemes, we consider the number of biochemical concerns that must be addressed. Using these biological self-assembly concepts to their fullest also shows how we can add complexity on a large-scale.

A. DNA transistor

The core members of the Israeli group at Technion are Dr. K. Keren, then, a doctoral candidate with a biochemistry background, and Dr. E. Braun, a physicist. Together, Dr. K. Keren provided biochemical insight on how to usefully bridge the gap between biology and nanoelectronics. Their research resulted in a handful of important concepts that effectively use biological self-assembly.

DNA is not a good conductor, so we must alter DNA to gain control over its electrical characteristics. But metallizing every strand of DNA in a scaffold would just short-circuit any DNA-based device, or externally-positioned non-DNA-based nano-device. One of the things we want to be able to do is metallize DNA selectively. Since base pair sequence is a way we can engineer molecular accuracy into nucleic acids, a method using bp sequence would be optimal.

Thus, the breakthrough work at Technion began with the achievement of sequence-

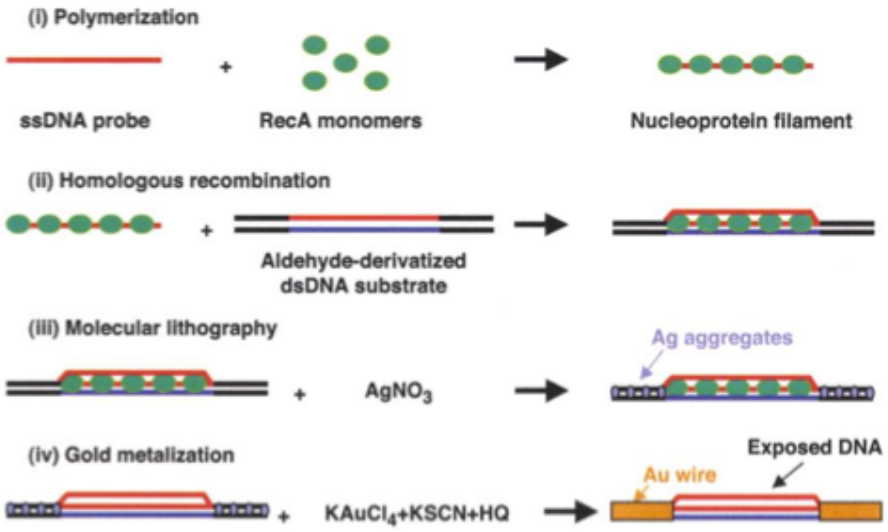


Figure 35: Method for sequence-specific molecule lithography on dsDNA. The phases of the experiment are (i) polymerization, (ii) homologous recombination, (iii) molecular lithography, (iv) gold metallization. (i) Polymerization is where the RecA-ssDNA probes are made, (ii) homologous recombination refers to when the dsDNA and the RecA-ssDNA forms a triplex, (iii) molecular lithography primes the "uncovered" dsDNA with Ag aggregates, and (iv) gold metallization completes the metallization process. [69]

specific one-dimensional “lithography” on λ-DNA [69]. Concepts explained earlier are used extensively in this method (see Chapter 3, Section D, Homologous Recombination for more). A 2027 bp segment of the λ-DNA (dsDNA) was chosen to serve as the insulating gap: a complementary strand to this segment became the ssDNA “probe” (see **Figure 35**). Applying the protein RecA (used in homologous recombination) to that piece of ssDNA helped define the sequence-specificity of RecA polymerization. When the solution of ssDNA-RecA strands (the nucleoprotein filaments) is mixed with linear molecules of dsDNA, the RecA helps form a triplex with the dsDNA. The RecA-ssDNA probe attaches only to segments of dsDNA containing the bp sequence complementary to the targeting ssDNA strand. The segment of RecA-stabilized DNA triplex is called the “homologous site”. The polymerized RecA spirals arund the ssDNA-dsDNA triplex bundle, protecting the DNA strand from metallization, like a negative photoresist. In other words, the ssDNA segments must be the “negative” of the sections we would like to metallize. The un-metalized segment effectively makes an insulative gap (see **Figure 36**) between two segments of DNA that behave more like a metallic wire [69]. This technique effectively allows us to use our control over the base-pair sequence to control conductivity.

This method of molecular lithography on dsDNA offers us a number of new benefits. The use of ssDNA probes provides single-base accuracy, in other words, control over every 0.3 nm of the strand. Apparently, the RecA binds the triplex together so tightly that it prevents even small ions (Ag ions for this experiment) from accessing the dsDNA. And although their sample was a one-dimensional strand of DNA, applying the concept of homologous recombination to a 3-armed DNA junction could take this concept a step further. RecA can stabilize a 3-arm DNA junction, although the results of metallizing such a junction are not shown [69].

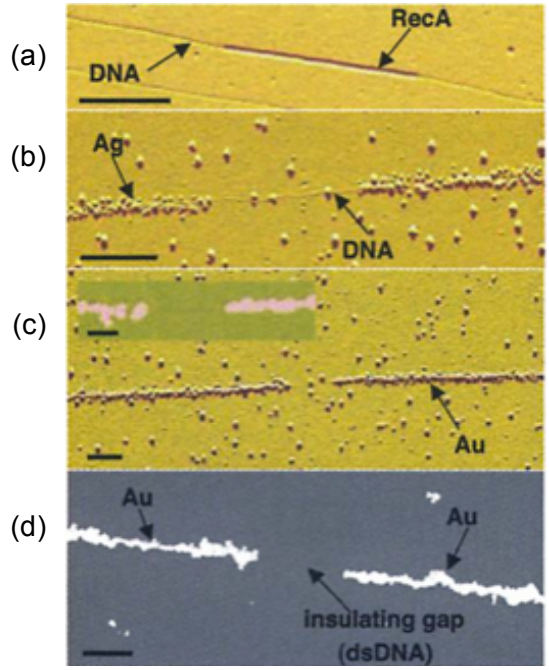


Figure 36: Results of the sequence-specific molecular lithography on dsDNA. (a) AFM image of the 2027-bp RecA-ssDNA probe attached to the λ-DNA. (b) AFM image of sample after molecular lithography (Ag aggregates). (c) AFM image of sample after gold metallization (scale bar in inset, 0.25 μm). (d) SEM image of wire after gold metallization. All the scale bars show 0.5 μm [69].

Assuming a device based on just DNA and metal conglomerates is insufficient, we must consider how to position nano-elements on dsDNA. Out of all the nano-elements we discussed in Chapter 2, carbon nanotubes probably have the most promise as a molecular

wire. The DNA-CNT transistor described in the Chapter intro, before this section, used both biotin and streptavidin to guide the placement of the CNT on the DNA strand. For more on biotin-streptavidin, refer to the end of Chapter 5. For this method, antibodies are used to help conjugate biomolecules, or attach them to each other.

For this experiment, we interrupt the same process as the last experiment, by placing the CNT before metallizing the dsDNA [68]. Two antibodies attached to each other in the homologous site readies that area for CNT-localization. One antibody directly attaches to RecA, while the second antibody is functionalized with biotin. Streptavidin readily adsorb onto CNTs, making the nano-element biologically recognizable to biotin. So mixing the streptavidin-functionalized CNT and the biotin-functionalized RecA triplex together localizes the CNTs on the DNA in a semi-precise way (see **Figure 37**). The paper makes no note on whether the streptavidin-biotin is actually removed as suggested by the diagram, and neither does it mention how the streptavidin-biotin complex affects the conductivity of the end device. Nonetheless, when RecA or another protein preoccupies the DNA's base pair sequence, using biotin and streptavidin gives us another way of localizing nano-elements.

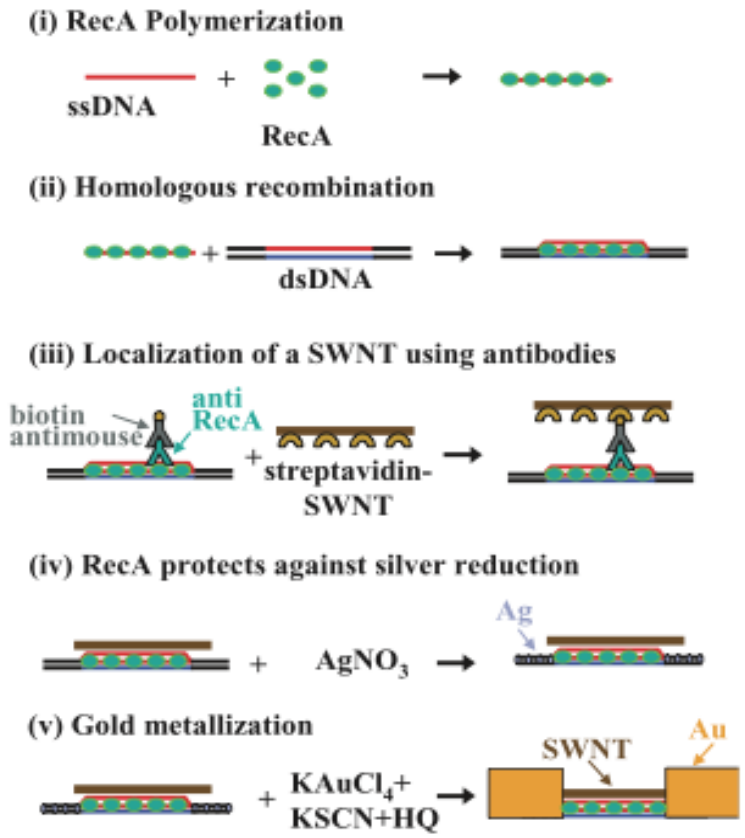


Figure 37: Method for sequence-specific molecule lithography on dsDNA. Addition of a step between the homologous recombination and the molecular lithography step where RecA protects the dsDNA by forming a triplex, allows the localization of CNTs between dsDNA [68].

Let us take inventory over the new tools we now have in our "DNA toolkit". A DNA-binding enzyme could do any of a wide variety of things with DNA: it can cleave DNA, it can copy it, it can attach two pieces and so much more. Scaffolds can also be made out of DNA using these enzymes and the concept of "sticky ends". Using sticky ends translates our control over bp sequence into an ability to localize nano-elements, and can help us place nano-elements on a scaffold. We can even change the conductivity of DNA by selectively metalizing it using RecA, in a

sequence-specific manner. To functionalize the insulating gap that results from this procedure, biotin-streptavidin affinities can be used to localize nano-elements onto the homologous site, where the base-pair sequences are already involved in the DNA-RecA triplex. This brings us one step closer to having molecular-level control over conductivity. Now that we have increased our DNA-based repertoire with these methods, our DNA toolkit might have everything it needs.

But before we assume these advantages and techniques are all we need, let us assess their limitations. In bringing biological self-assembly and nanoelectronics together with these methods, we seem to make a tradeoff with reproducibility. In the gold metallization chemical process, the chemistry caused the Oswald ripening of the metallic nano-clusters that grew to microscopic proportions [69]. Such coarse chemical processes metallized DNA past the point of biological recognition [70]. This presents a problem to our more important goal of using biological self-assembly wherever possible. We lose the ability of using DNA-modifying enzymes any further by this style of metallizing DNA. Even the method of applying CNTs was not perfectly reproducible: sometimes CNT ropes formed (see **Figure 38**). CNT ropes have completely different conductivity properties from CNTs. For both situations, the metallization scheme rendered DNA-templated wires too coarse to make fine contact with nano-scale devices in a reproducible manner. Thus, reproducibility is the Achilles heel of this.

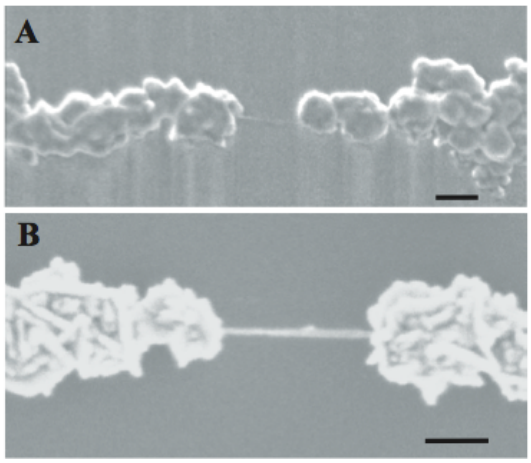


Figure 38: SEM imagines of DNA-templated CNT devices and the metallic "wires" contacted to them. (a) single CNT; (b) a rope of CNTs. Scale bars show 100 nm [70]. Remember that the CNT ropes conduct metallically, whereas the conductivity of single CNTs depend on their chirality (Chapter 2, Section A).

But how to do we coordinate the above methods with the ability to make scaffolds out of DNA? It is uncertain what order we should carry out these processes, chemically. Should scaffolds be made out of the DNA first, and then metalize the DNA strands? Or should would metalize the DNA first and then assemble them into scaffolds? If we had to metalize the DNA first, and then make scaffolds, biological recognition even after metallization is necessary. In other words, the backbone of DNA has to be accessible for DNA-modifying enzymes used later.

If we could selectively metallize DNA without compromising biological recognition, then we would be in business again. We already know that RecA binds so completely to DNA that it can block out even atomically small ions. So we need a new metallization scheme. M-DNA gives us a way to metalize DNA without covering DNA's backbone.

B. M-DNA

Contrary to B-DNA (typical DNA), M-DNA is a DNA molecule complex that conducts electricity. Here, "M" stands for metal-containing, because in M-DNA, polyvalent metal ions, i.e. Zn^{2+}, Co^{2+}, Ni^{2+}, and more, exist along the center of the DNA helix [45]. The metal ions "interrupt" the hydrogen bonding between the base pairs (see **Figure 43**). The imino proton is the name given to the hydrogen atom that the metal ion replaces [71]. As part of the base-pair stacks, small 0.4 nm gaps separate the metal ions. Effectively, this creates a semiconductor that is only about 2 nm thick, and can be classified as a self-assembling molecular wire. This is different from G-4 complex whose formation of that complex was facilitated by cations (see **Figure 31**). The metal ions in G-4 did not interrupt hydrogen bonding and removed easily because they were not part of the base pair stacks. On the other hand, the metallic ions in M-DNA are inserted in the same plane as the base pairs, and can hence be considered an integral part of the greater DNA molecule. Because three hydrogen bonds are formed between *GC* base pairs, versus the two formed between *AT* (see **Error! Reference source not found.**), B-DNA with higher *GC* content is expected to form more stable M-DNA complexes [72]. To distinguish between the metallic DNA and DNA's normal form, we use the term "B-DNA" to make it absolutely clear when we are talking about regular DNA in this section.

On a greater molecular level, to see how metal ions fit into the greater structure of B-DNA, see **Error! Reference source not found.**. The chemistry of this kind of molecule is expected to be similar to B-DNA for the most part, except for its stability in more basic environments. Converting B-DNA to M-DNA slightly alters the geometry of the B-DNA. Results from mathematical models show that changes in the angle of hydrogen bonding is usually less than 5°, and the hydrogen bond length usually is reduced by 0.008 nm (see **Figure 40**) [73]. The biological systems, the purpose of M-DNA is to make some DNA less amenable to enzymatic

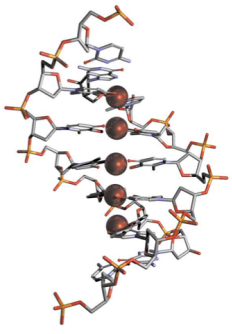
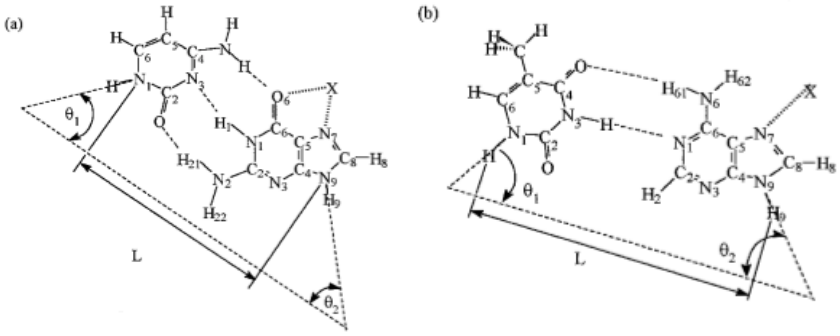


Figure 40: M-DNA molecule. Stick model of B-DNA segments and red balls represent metal ions, which could be of any valence.[71]

Figure 40: Definition of angle of H-bonding and H-bond length. Changes in these parameters in Watson-Crick pairing are measured using nonempirical ab initio methods. (a) guanine-cytosine (*GC*), (b) adenine-thymine (*AT*).[73]



modification: this is useful in biomedical engineering [74]. But concrete empirical evidence is needed to definitively know which enzymes can bind to the backbone of M-DNA.

Bonding between divalent metal ions (like Zn^{2+}, Co^{2+}, or Ni^{2+}) and B-DNA results in M-DNA at pH > 8, depending on the cation (positively charged ions). The cation literally replaces the imino protons of the base pairs, coordinating with them as per their oxidation state, when M-DNA is formed (see the "H_{21}" in **Figure 40**(a) and "H" on N_3 in **Figure 40**(b); compare to **Figure 43**). Although *GC* content was predicted to be an indicator of M-DNA stability [72], B-DNA with any sequence can form this structure in a generally stable manner, regardless of *GC* content [74]. The rate of formation strongly depends on temperature, pH, and cation concentration. For example, it takes 30 minutes to convert half of the B-DNA in solution to M-DNA at 20°C, a pH of 8.6, and 1 mM Zn^{2+} [74]. This is reported to work with Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Cu^{+}, Ag^{+}, Au^{+}, Zn^{2+}, Cd^{2+}, Hg^{2+} and many other metal cations [73], [75]. But metal cations that have a high affinity to DNA do not necessarily bind to B-DNA in the right way. For example, Mg^{2+} has a high affinity for DNA, with $K_a = 2 \times 10^5\ M^{-1}$ or $K_d = 5 \times 10^{-6}$ mol/L. But Mg^{2+} binds more readily to the phosphate groups in DNA than settling into the base pairs, versus other Mn^{2+} and Cu^{2+}, with whom nucleobase coordination is a little more likely.[72] Some of these ions have been analyzed for their binding preferences: $Co^{2+} > Ni^{2+} > Mn^{2+} > Zn^{2+} > Cd^{2+} > Cu^{2+} > Ag^{+} > Hg^{2+}$ is the order of preference for phosphate over nucleobase coordination [76]. So we also need to choose metal ions based on this phenomenon as well. Using synthetic base pairs (not *A*, *T*, *G*, or *C*) can lower the pH at which formation occurs. In particular, the lower the M-DNA will form at a lower pH if the pK_a of the base's imino proton is lower [77]. But overall, the big-picture benefit this technique offers is that M-DNA could give us a way to tailor the conductivity of a DNA-based molecular wire.

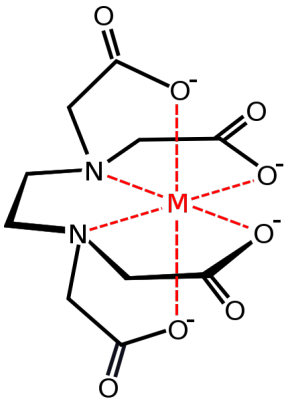


Figure 41: Metal-EDTA chelate. The metal ion can make up to 6 bonds with EDTA. The total complex is called the "chelate", which is very stable.

Removing the metal ions from between the base pairs is also controllable: lowering the pH of the solution or removing the metal ions can convert M-DNA back to B-DNA. Something that binds to metal ions more strongly than the base pairs in B-DNA can remove the metal ions from the DNA structure. For such applications, ethylenediaminetetraacetic acid (ETDA) is a commonly used agent for metal chelation (chelation refers to the removal of metals in a biological context). EDTA is a poly-amino carboxylic acid that uses its oxygen and nitrogen atoms to bind to a metal ion that could have any oxidation state (see **Figure 41**). For a given strand of B-DNA, the metal ions used, the base-pair sequence, and the solution all affect

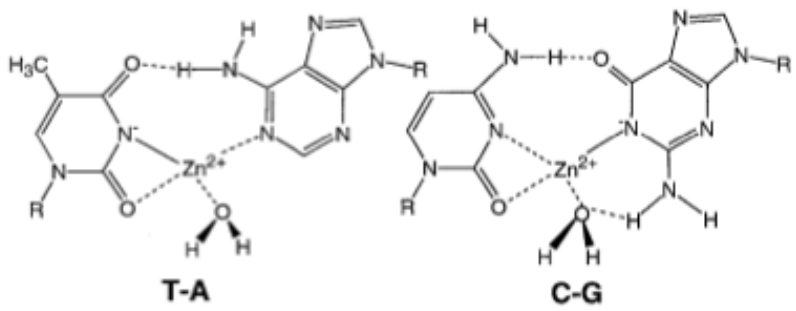


Figure 43: How metal cations "interrupt" hydrogen bonding in M-DNA. "R-" stands for the sugar and phosphate groups in the backbone of regular DNA. The dotted lines show where the H-bonding happens, for the *GC* and *AT* base pair cases. Here, the cation Zn^{2+} is assumed, but the same relationship could be shared with any other divalent cation. The metal replaces the imino proton.[77]

the hysteresis of M-DNA conversion and B-DNA reconversion [77]. So if need be, experimentally, we can convert M-DNA back to B-DNA for DNA modification purposes.

Fluorescence quenching shows that this molecule can conduct electricity like a molecular wire [75]. Such measurements may suffice for organic solar cell applications. But, beyond that, this analysis may be insufficient for our nanoelectronic purposes. Conductivity measurements similar to experiments conducted for for B-DNA bundles were conducted on M-DNA as well [78]. The measurement was taken between a 15-μm physical gap between two metal electrodes in a vacuum. The M-DNA bundles, made with Zn ions, behaved like a metal when placed over that gap between the contacts. Compared to the B-DNA behavior in the same setup, where the I-V curve plateaus for 0.25 V before rising, M-DNA exhibits no plateau in the I-V curve (see **Figure 42**). But we know that we need more precise measurements than that of M-DNA bundles. Even ab initio nonempirical approaches may not suffice because physicists cannot oversimplify the M-DNA molecule by modeling it as an infinite single atomic system of metal cations. The metal ions being used are cations: they do not have their valence electrons because they have been stripped of them already. So its uncertain about whether the metal cations still contribute to B-DNA's conductivity as metals, without those contributing electrons.

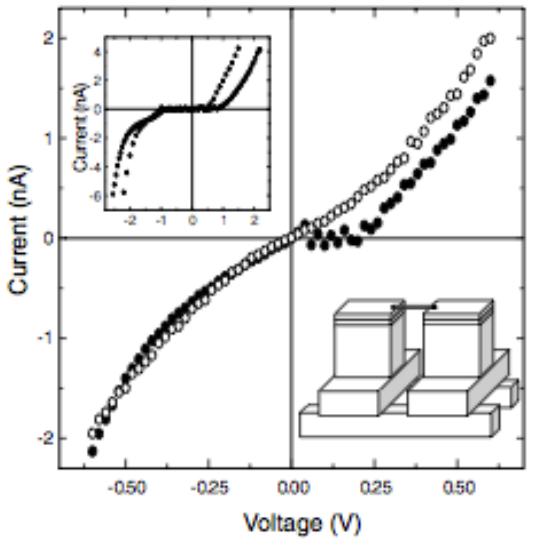


Figure 42: I-V curves measured in vacuum at room temperature. Results for M-DNA (O) and B-DNA (●) shown. Upper inset shows two representative current-voltage curves measured in vacuum at room temperature on samples with Au–oligomer–B-DNA–oligomer–Au in series [78].

Using M-DNA, a laboratory-scale FET transistor has also been constructed to show M-DNA's nanoelectronic promise [79]. This FET operates on a slightly different principle because the rest of B-DNA surrounds each metal ion. The B-DNA structure can be considered an "asymmetric ligand field", and its helical nature means that each

adjacent metal ion has a different structural environment. The adjacent metal atoms have different energy levels, impeding the hopping of electrons through M-DNA. Like a conventional transistor, significant modulation occurs in the ±10 V range. But unlike a conventional transistor the conductivity decreases when a positive bias is applied and when a negative bias is applied. The paper had no graphs resembling the I-V curve of a conventional transistor, so the paper's resulting diagrams are not included.

The benefit of using M-DNA is that this definitely gives us the opportunity of tailoring the conductivity of B-DNA to our needs. Ideally, choosing metal cations with different oxidation states could make B-DNA that is even more concretely n-type and p-type. Perhaps using the B-DNA lithography method with RecA would allow us to selectively metallize strands of B-DNA. Since the backbone would remain unaffected by the metal ions, hopefully this process could be applied progressively. By that, I mean that Co^{2+} ions could be applied before Cu^{+} ions are applied and so on and so forth. Working out this chemistry will obviously be critical to the successful application of this method. Moreover, B-DNA can be made into scaffolds. But let us not get too ahead of ourselves.

There are still unresolved issues associated with the application of M-DNA to our purpose. When we choose metal cations, we need to worry about whether it will actually form M-DNA by binding to the base pairs instead of binding to the backbone. There are no concrete measurements on conductivity of single-molecule M-DNA yet. Also, there are no studies proving that enzymes can bind to M-DNA backbone, thus far, so this must be investigated. The ability of a given enzyme (or protein) to bind to M-DNA will probably be related to the pH range that it can function in, because M-DNA is only stable in basic conditions. So if there are any problems with compatibility between a molecule and a protein, one will have to be reconsidered or modified. These aspects of M-DNA must be investigated, but for now let us consider that they are minor issues that have existing solutions. We postpone the resolution of these issues because of the unsurpassed benefits that B-DNA provides. This way, we can plausibly illustrate the opportunity that the B-DNA-based concepts introduced in this chapter provide.

Regardless of the aforementioned issues, B-DNA gives us a way to make three-dimensional scaffolds. So using B-DNA is the only way to meet our biological self-assembly standards. With this foray into chemistry, biochemistry, and other biology concepts, we were hoping to gather tools that would help us find a way to mass-produce an array of working nano-transistors. Now with these new concepts, B-DNA lithography with the protein RecA and metallizing B-DNA to make M-DNA, we might have everything we need, in a way. We have a nano-scaffold that can self-assemble, on which we could position electrically functional nano-

elements. But because the nano-elements we considered have anti-complexity issues in their implementation, we turned towards B-DNA and its electrical properties. Although B-DNA is a terribly unreliable conductor, we can turn it into a good conductor (if M-DNA really works the way we want it to). With B-DNA lithography, we have a way of metallizing B-DNA selectively, using base pair sequences to control conductivity in a location-specific way. But, the question remains: how do we bring it all together to actually make functional transistors in 2D or 3D space? Let us see a theoretical example of how these concepts can come together to do that.

C. A plausible scheme for mass synthesis of DNA-based transistors

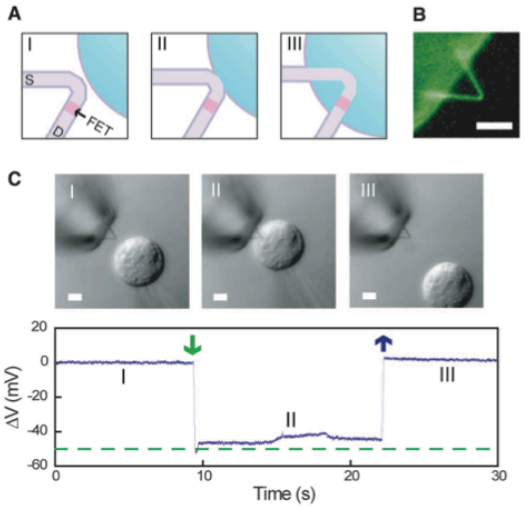


Figure 45: How the FET can be used as a biosensor. (A) Schematics of nanowire probe entrance into a cell. The purple outline represents the phospholipid bilayers, and blue represents the innards of the cell. (B) False-color fluorescence image of a lipid- coated nanowire probe. (C) Differential interference contrast microscopy images (upper panels) and electrical recording (lower panel) of an HL-1 cell and 60° kinked nanowire probe as the cell approaches (I), contacts and internalizes (II), and is retracted from (III) the nanoprobe. The dashed green line corresponds to the micropipette potential. Scale bars, 5 μm [80].

From here onward, DNA refers to the "B-DNA" of the last section. The concepts of three papers are key in this: the three-dimensional nano-scaffold based on the tensegrity triangle[37] (Chapter 4, end of Section B), DNA lithography using RecA[69] (Section A of this chapter), and M-DNA [71]. The purpose of this section is to answer the question, "how can M-DNA be manipulated into a form that could act like a transistor?" We make a few assumptions to show a plausible scheme. Firstly, we assume that the DNA lithography using RecA is completely compatible with the formation of M-DNA. We also assume that we can alter the bp sequences of some of the tensegrity triangle's legs in the nano-scaffold, without sacrificing the overall ability to self-assemble the scaffold. We also assume that the RecA-ssDNA strands do not polymerize contiguously over corners of the triangle: I will explain this requirement shortly. In the rest of this section, we will see how these assumptions are key to making this idea work.

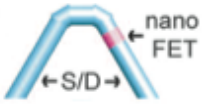


Figure 44: How 2 kinks and doping can make a FET out of a Si NW. The blue and pink regions designate n-regions and the p-region in the npn FET, respectively [80].

The geometric inspiration of this transistor comes from the same Harvard professor who was working on silicon nanowires. The Lieber lab made and tested a FET based on nano-wires, and suggested how it may be used as a biosensor [80]. Varying reactant pressure during nanowire growth can introduce a 120° kink in the nanowire being grown. Putting

two 120° kinks in the nanowire right next to each other can give the overall nanowire a very precise 60° "V" shape (see **Figure 44**). If the "V" of the transistor were a fixed distance from a substrate, this may act in a transistor-like fashion. In-situ doping during nanowire growth can form an "npn" transistor on part of the "V" shape. A probe is designed from this "V" shaped wire by attaching it to a bulky but flexible modules with electrical interconnects on it. To use this device as a biosensor, after the wire's surface is modified with phospholipids, moving the tip in and out of the cell changes the voltage recorded macroscopically (see **Figure 45**). The main takeaway from this paper is that this geometry could work for our purpose, if we can somehow make this shape with DNA.

Fixing the "V" of the probe at a fixed distance from a second straight wire, without touching it, would result in a hybrid of the crosswire concept and the aforementioned concept. The papers by Lieber lab verifying that those concepts work in practice also serve as proof that this concept would work. As long as some sort of insulative material were dividing the "V" from the lower line, then the "V" might work as a gate, and the two ends of the lower wire may behave like source and drain. This is distinctly different from the concept of the biosensor [80] because in the "V"-shaped probe, an npn transistor is embedded in that wire. In this case, the FET-like behavior would instead arise from the separation between the two wires. Let us see how this geometry may be possible using DNA.

If we begin with the unit cell of the three-dimensional nano-scaffold, the tensegrity triangle, this has three possible "tips" on it, where the legs of the triangle are extended. Extending the top two lines of the tensegrity triangle as it was drawn schematically in Seeman's paper, could make the "V" of our nano-transistor (see **Figure 46**). The line below that "V" would play the role of the source and drain. Selectively metallizing the "V" and the line, and leaving the middle part as non-conductive DNA would result in a transistor-like device, according to Lieber.

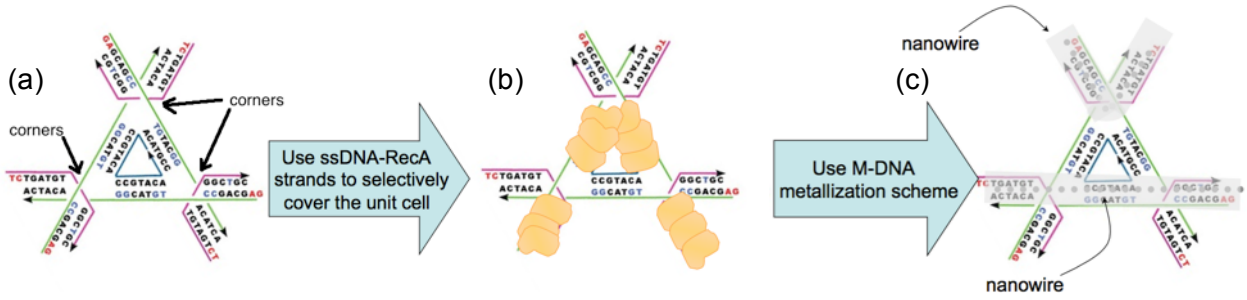


Figure 46: A suggestion of how to use of DNA lithography and M-DNA to make a nano-electronic device. The yellow globules in the second schematic represent where the ssDNA-RecA strands polymerize. In the third schematic, the strands of DNA shaded gray represent the M-DNA. This scheme assumes that (1) the using RecA is completely compatible with the formation of M-DNA, (2) we alter the bp sequences of the legs of the tensegrity triangle that are covered in RecA, (3) the RecA-ssDNA strand can polymerize contiguously over corners of the triangle, and (4) partially metalizing the DNA doing so does not sacrifice the overall ability to self-assemble the scaffold.

To make “nano-wires” in this geometry, first of all, we would need to make RecA polymerize to ssDNA, resulting in nucleoprotein filaments with homologous sequences to the four segments (see **Figure 46**). These four segments are the portions of the DNA we want to protect with RecA and thereby prevent from conversion into highly conductive M-DNA (i.e. the four segments depicted as being covered by the yellow blobs of RecA in **Figure 46**b). For the triangle at the very center of the figure (b), two legs of this triangle would have to have different bp sequences so that the RecA-ssDNA probes will bind to the two of them and not the third side of the triangle. The bp sequences of the DNA extensions below our source and drain would need to be reassigned as well. After making the nucleoprotein filaments, the filaments stabilize triplexes containing homologous sequences by unidirectional branch migration. The branch migration is facilitated by ATP, and continues insofar as the RecA-ssDNA strands are *not topologically restricted*. But because the scaffolds are small and are most likely topologically restricting, the corners (the intersection in the Holliday junctions involved, see **Figure 13**) will not be covered**.** This lack of RecA corner coverage works to our advantage at the top of the device's central triangle in that it means that the "V" coming from above it (our device's gate) will be free of RecA at its tip, and thus this whole "V" will be converted to conductive M-DNA. The net result would be a completely self-assembling linear wire, with a conductive V spaced precisely away from it by the still-insulating two legs of the central DNA triangle.

In essence, what we have is a conducting “V”-shaped gate adjacent to a semiconductor straight channel (see **Figure 47**). Such a configuration would work like a transistor when the “V”-shaped part is supplied a voltage as if it is the gate and the ends lower wire are doped to act like source and drain. Sufficient evidence points toward why this behavior is likely: in particular

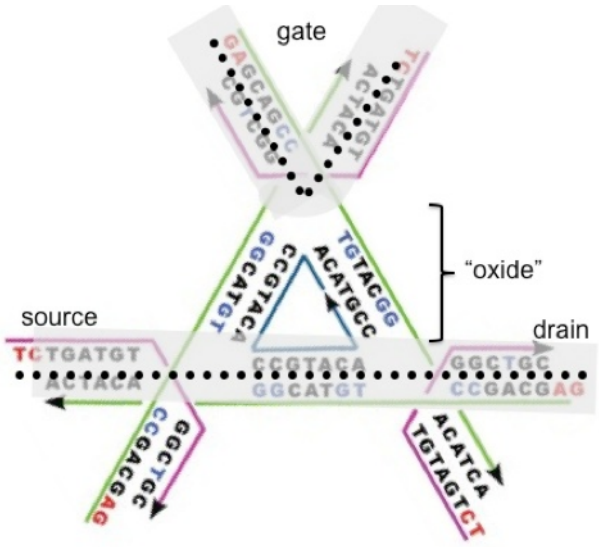
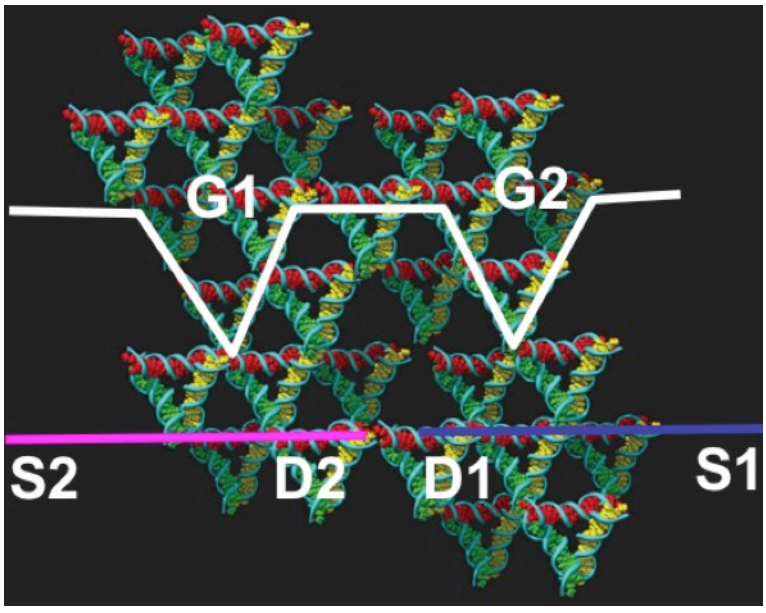


Figure 47: Diagram of DNA-FET idea showing “gate”, “source”, and “drain”. The two legs of non-conducting DNA would behave like the oxide, separating the gate from the source, channel, and drain. The DNA with the black-dotted overlay represents the segments of DNA that are converted into M-DNA. The upper “V” wire acts like the gate. The lower linear wire, when doped properly, acts like the source drain and channel. And the DNA struts separating the wire material act like the oxide.

Lieber's work with the cross-wire FETs [81], and his work with the NW cell-probes [80]. As a voltage applied on the V-shaped gate (see **Figure 47**) changes above the source and drain, the current traveling through the channel would be changed in the same way it would for a cross-wire FET [81] or the NW cell-probes [80]. What works in our favor is that M-DNA, unlike DNA, is a zero band-gap material (see **Figure 42**) [75],[78]. This gives us ample evidence to believe that M-DNA is indeed a material whose electronic properties tend to be metallic in nature. Certainly the electrical characteristics of the NW and M-DNA are different, but because both are conductors, the same principles should apply.

Because no concretely electrically conductive things connect the "V"-shaped part to the lower nanowire, the working principle of this structure bears resemblance to the crosswires of Lieber's FET. If you remember from Chapter 2, Section B, the crosswire depended on two silicon nanowires that would crossover each other without touching. To ensure that the two un-metallized legs of DNA behave like an insulator, other less electrically conducting proteins could be employed. Just like the gate can toggle what mode the channel is operating in for a traditional FET, the "V" part of this transistor would have some type of control over the conduction in the M-DNA channel.

To make this idea work better a few precautions could be employed. First of all, since the base sequences of the two inner legs need to be changed anyway, so that the RecA binds to both of them and not to the third leg, if that sequence contained no guanines then those legs would be even less conductive. Furthermore, M-DNA can be made with metals of different valence states: this could give a doping effect to the source-channel-drain wire.



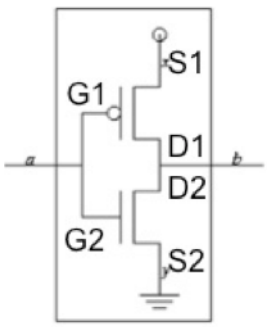


Figure 48: How a NOT gate is possible in a 3D scaffold using this idea of a DNA-FET. The part in black shows what wires would be rendered as M-DNA in a 3D tensegrity triangle scaffold. The part with the white background shows the sources, drains, and gates of the effective n-MOS and p-MOS.

Note that the aforementioned unit cell repeats to form a greater network of DNA in the form of a scaffold with nano-scale. So even a NOT gate is possible with this geometry because the scaffold continues in all three directions. To make effectively an n-MOS and a p-MOS the source-channel-drain wires would have to be doped in opposite ways (see **Figure 48**). In the diagram, the fuschia and the dark purple line represent how those wires would need to be doped differently or treated differently to get a n-type behavior and p-type behavior like an n-MOS and p-MOS. This is why the scaffold is important: it is what literally takes us from chemistry to the circuit-level.

D. Achieving a functional nucleic-acid based device

What makes all of this is possible is choosing to employ biological self-assembly for nanoelectronic purposes. This, and more discoveries in interdisciplinary fields bridging electronics and biology, can be used to contribute to the fabrication of massive arrays of functional nano-transistors. The continued integration of ideas, and employing biological tools for the nanoelectronics toolkit, can lead to further possibilities, which go far and beyond this idea. In realizing this idea, for example we would probably try to make a flat device in a two-dimensional DNA scaffold. Once such a laboratory technique would show technological promise, the three-dimensional DNA scaffold would be conquered, and so on and so forth. This implies how making vast arrays of devices, nano-sized contacts, nanowires, and nano-scale interconnects are possible now with this approach. We now have tools to functionalize, position, and wiring together future nano-electronic circuits.

On a broader conceptual level, let's look at the pieces that make this idea work. First of all, we have a nano-element that self-assembles AND can be selectively turned into a wire-like material. The self-assembly characteristics gives the ability to make scaffolds out of that nano-element, scaffolds that act like a framework for the devices, interconnects, etc. Any nano-element capable of forming a scaffold in a logical, possible manner, may take this approach of building a circuit. The scaffold needs to be made of non-conducting material such that it can be converted into conducting sections (as this method shows) or vice versa. So inter-conversion is a necessity too. Secondly, we need a nano-wire geometry that works electronically. Here, a same-only-smaller approach may or may not work. Either way, a geometry that uses the quantum effects at play such that the device's IV-curve exhibits leveled saturation. Once that geometry is determined, a scaffold is needed that somehow involves that geometry. The scaffold's geometry should be such that the selective metallization technique can take the scaffold the rest of the way.

I think, the root of the strength of this technique is that how we have location-specific control *and* control over the conductivity of the material on the nano-scale. Once those two hurdles are championed, figuring out a way to use quantum effects to our advantage, to achieve the right IV-curve, would take precedence. The geometry of this idea may or may not work, because there are complications with DNA's conductivity. But the novelty in this idea is not in whether this configuration works, but it shows how these ideas need to come together to achieve the visions embellished on in Chapter 1. This is only one way the ideas out there could come together. Self-assembly in biology could alternatively make electronic organisms too, a possibility that will be explored in Chapter 7.

Admittedly, such schemes for self-assembly-based nano-electronics may never replace silicon-based technology in its totality, but it independently has a number of pertinent applications. The applications of the resulting devices and circuits may not initially fit the purposes of rugged large-scale-integration nanoelectronic systems. This is because the first prototypic devices will probably not have the robustness that device engineers look for in MOSFET-replacement devices. But, integrating these kinds of ideas could help make molecular electronic, or plasmonic, circuits. Because such a transistor would be based on nucleic acids, a type of organic material, this research impacts the fabrication of nano-scale, biocompatible bio-probes. Such nano-scale bio-probes were not possible before because silicon-based technologies face such serious biocompatibility issues. But they might be better adapted for the sake of biochemistry, because making probes made out of organic materials are less likely to be rejected by the body. Power dissipation

But the implementation of this design is definitely more involved than the aforementioned proposal. For example, if M-DNA really works like a nanowire with excellent electrical conductivity, this may or may not work like a transistor due to fringing effects of the electric field, quantum effects that could take over, and other counter-ion related problems. Also, this is in some ways a same-only-smaller sort of geometry, so a transistor geometry that actually exhibits the right IV-curve would look completely different because of quantum effects. But even if this structure does not work like a transistor, attempting to integrate biological self-assembly concepts with nanoelectronic purposes will be undoubtedly beneficial to nanoelectronics.

In the next chapter, we explore the break down of some of the other assumptions we have made to illustrate the requirements for the success of this design. Some detours into other fields of biology may prove useful in making this idea a reality. The following chapter explores these subjects.

- ❖ -

Picture references:

- Metal-EDTA complex bonding (http://en.wikipedia.org/wiki/Ethylenediaminetetraacetic_acid)

CHAPTER VII.

Future Research

Integrating biological self-assembly with the device physics and materials science already present in nanoelectronics can show how we can add complexity in more feasible ways. Biology concepts can endow us with the ability to place nano-elements accurately, and control conductivity in a location-specific manner. The previous chapter illustrated how using these biological self-assembly concepts to their fullest can indubitably benefit nanoelectronics.

In the last chapter, the success of the technology proposed relied on certain assumptions. They were:

(1) the RecA-ssDNA strands do not polymerize contiguously over corners of the triangle,

(2) the use of RecA and M-DNA formation are compatible, and

(3) changing the bp sequences of tensegrity triangle legs do not compromise the ability of the strands to self-assembly into a nano-scaffold.

The first assumption, in fact, holds true according to literature: ATP hydrolysis facilitates unidirectional branch migration if RecA-ssDNA strands are not topologically restricted [82]. And since the corners of the tensegrity triangle geometry are in fact really tight, on the nano-scale, we can be quite sure that RecA will not cover the corners. This is important to that design since we want the tip of the "V"-shaped gate to form. But the other assumptions break down, opening up paths of future research in search of solutions, in other areas of biology.

For example, upon deeper inspection, considering the effects of pH is important to gauge the compatibility of the use of RecA with the formation of M-DNA. It turns out that these methods are not compatible due to pH, and there are two ways of addressing this: either by altering the protein (using protein engineering, to change the pH at which it is stable), or by changing the substrate (in other words not using DNA). Because if RecA cannot work with DNA, then we would have to consider whether the ssDNA-RecA probe could homologously attach to other nucleic acids. Using a conductive polymer would be optimal to try to achieve conductivities that are closer to that of silicon-based technology's materials. Also we do not want to sacrifice the ability to self-assemble, so we must consider everything.

To alter the bp sequences of some of the tensegrity triangle's legs in the nano-scaffold, without sacrificing the overall ability to self-assemble the scaffold. Investigating the effects of such alterations would be a matter of experimental expertise best known to the Seeman lab and similar research laboratories.

A. Possibilities in protein engineering

Although pH is something we overlooked when discussing the integration of ideas in the last chapter, it matters to the proteins that we apply to DNA. Because pH can affect proteins chemistry, and how they work, considering pH is important in determining if the methods for using RecA and making M-DNA are compatible. Otherwise, when forming M-DNA, the RecA covering parts of the tensegrity triangle might just fall off of the DNA. A photolithography analogy would be a process of metallization or doping that effectively rips the resist off. This, of course, would defeat the purpose of lithography, even on DNA.

Unfortunately, M-DNA formation and RecA usage do not work together because of the pH ranges these two molecules are stable in. M-DNA forms and is stable in the pH range of (pH ~7.5 to 9) [82]. Binding of RecA to dsDNA depends highly on pH: it is optimal at a pH of 6.0 and is undetectable at pH ≥ 7.5. This means that after RecA-ssDNA strand is polymerized on dsDNA, if we attempt to turn parts of that DNA molecule into M-DNA, we would accidently rip off our "resist" and just metalize everything. We need a protein that can withstand the pH range that our new metallization scheme requires. If we could modify RecA so that it *would be* stable in the pH range that M-DNA is stable in, we would be all set. In fact, we are not even limited by the aforementioned proteins. Even if we found a protein that stabilizes DNA triplexes the same way M-DNA does, and is stable in these basic pH ranges, then we would be back in business again.

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Protein engineering is an entire field of its own, offering methods of synthetically modifying the libraries of enzymes that already exist. Two ways of modifying proteins is (1) rational protein design and (2) directed protein evolution. Rational design, directed evolution, and combined approaches have successfully been used to improve biocatalysts for many applications. Protein engineers regularly use them to better the thermal stability, their tolerance towards salts, and even *their pH activity or stability*. These methods have been used to alter proteins for the sake of better biofuel cell design in German university labs, and related work illustrates how protein engineering may be applicable to the development of nucleic-acid-based molecular electronics. Let us look into these protein-engineering techniques to see how applicable they are to this research situation, and investigate their limitations [83].

Rational protein design is incumbent on the knowledge, or a fairly good model of the given protein's structure. This is the realm of computational biologists, which depends on predictions based on previously built models. Thus, it requires a deep understanding of the property that needs improvement and how it relates to the primary structure of the protein: this

is called the structure-function relationship. RecA's amino acid sequence is known,[84] but how it relates to its pH stability may not be already known, and would make for interesting future studies. Common alterations like thermal stability, activity, and selectivity are better understood in this field [[83]]. Computational biologists definitely understand other characteristics less (like electrical conductivity), so prediction would not be as accurate. Also, changing the solution environment makes engineering more computationally difficult due to a lack of understanding and a lack of successful reengineering approaches. This includes adding organic solvents, changing pH, or having to predict the effect of salts present, so much of the work done with DNA gets complicated fast. Given any protein, building a working knowledge is important if rational protein design is the chosen technique of engineering. But if the existing theoretical framework does not provide a direct explanation of how to change the range of pH stability for a protein, it may not be the easiest way to re-engineer RecA.

The other technique for protein engineering is directed protein evolution. Directed evolution does not require an understanding of the structure-function relationship of the targeted property, but you must know what gene encodes for the property you are trying to change. And indeed, the gene in the bacteria *Vibrio cholerae* that encodes for RecA has been found [[84]]. Directed evolution experiments consist of iterating through cycles of the three steps: (1) generating diverse mutant libraries, (2) screening for improved protein variants, and (3) isolating the gene encoding the improved protein mutant. The proteins, made from various genes, are created and folded, or "expressed," inside simple bacteria called "expression hosts". The downside to directed evolution is that they are much more time-taking compared to rational protein design outcomes [[83]]. Nonetheless, directed evolution has become a widely accepted and broadly applied method in academia and in industrial biotechnology. Directed evolution works the best for proteins with known crystal structures: and the crystal structure of RecA is known too [85]. And changing the stability towards certain pH is often done with directed evolution. It is a great tool for computational biologists because directed evolution experiments can reveal the structure-function relationships for them. And when time is an issue, protein engineers try to use mixed experiments, employing site-directed mutagenesis studies and screening for a few variants (often <10). But expression hosts and current screening technologies limit directed evolution technologies.

So we may have to resort to a different protein, if M-DNA must be used in our process. And inverse approach using this same information would be to find a protein that binds to DNA and makes it more definitely electrically conducting.

Returning to the original scheme, if the use of RecA is preferred over the use of M-DNA, then some other molecule (that is either an analogue to DNA or otherwise) might be plausible.

B. Other nucleic acids & conductive polymers

We have two reasons of considering conductive polymers. First, if RecA and M-DNA are incompatible, using a nucleic acid that is a DNA analogue might be an option. Such a nucleic acid would work if it could form a triplex structure with the ssDNA-RecA probe. This depends on how RecA binds with a DNA duplex because other nucleic acids may not offer the correct backbone. If researchers are interested in making a new nucleic acid, conductive polymers might as well inspire the design of its backbone. And secondly, we admit that the conductivity of organic materials cannot beat the materials used currently in silicon-based technologies. But these concepts may still be worth integrating for the sake of finding a nano-wire that conducts well and can biologically self-assemble.

RecA with PNA and possibly other nucleic acids

It may be possible to metalize other nucleic acids in similar ways and thus converting them into a plausible conducting nanowire [[76]]. But the issue with using anything other than DNA is (1) how to control metallization in a location or sequence specific manner, and (2) how to make nano-scaffolds using that nucleic acid.

Take PNA, for example. Because PNA is initially neutral, perhaps this can be metallized in pH ranges that are more amenable to the use of RecA [[76]]. Apparently RecA-ssDNA strands can facilitate strand exchange in PNA and other nucleic acids as well [86]. In the event that researchers try to return to using PNA as the base material, based on these hopes, there are still multiple repercussions to that choice. First of all, the formation and conductivity of M-PNA have not been investigated. In literature, there are no known PNA scaffolds similar to the tensegrity triangle nano-scaffold. Perhaps most importantly, because PNA does not have the right backbone, so whether ssDNA-RecA strands can attach to PNA must be investigated. Because of the lack of prior work in this field, any spin-off ideas would be almost purely speculative. But most of all, our biological self-assembly standards are compromised with the use of non-DNA nucleic acids.

What maybe more likely is using PNA to make something DNA-based more possible. PNA may help RecA-ssDNA strands bind to dsDNA at higher pH [87]. But this is only for linear dsDNA, and dsDNA in the three-dimensional nano-scaffold is definitely anything but one-dimensional. So even this might not impact this research in such a way to make this idea more possible.

But in the case that an alternative nucleic acid is the way to go, we could embark on a quest for high conductance DNA, or nucleobases more conductive than guanine.

Conductive Polymers

To continue the discussion of conductivity from Section A of Chapter 5, another direction of future research is towards integrating concepts from the field of conductive polymers. Although organic materials are almost always less conductive than metals, exploring the concepts that guide transport in conductive polymers may also be useful. In organic conductive materials, charge transport happens by some combination of strongly distance-dependent tunneling and distance-independent incoherent transport (hopping). So to increase the conductivity of a material, we will be talking about methods used to make organic conductors more conductive. Perhaps this approach will provide us with the perfect bridge between nanoelectronics and biological self-assembly.

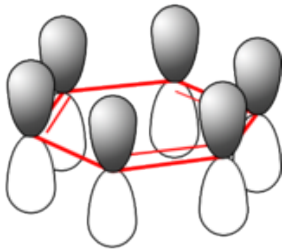


Figure 49: Continuous p-orbitals (p_z) of a benzene ring. This shows how the electrons are delocalized through out the ring.

Conductive polymers are classified by whether they contain aromatic cycles, alternating double and single, or both. In addition to the structure, the atoms that are neither carbon nor hydrogen atoms, also known as "heteroatoms", also affect their electronic character. The alternating single and double bonds are characteristic of "conjugated systems" in organic chemistry. The common characteristic of all these molecules is that they have a continuous chain of π-bonds (i.e. benzene, see **Figure 49**). The external addition or removal of an electron pair causes the double bond to either move over one to push over the adjacent double bond or fill in the adjacent lack of electrons (see **Figure 50**). In organic chemistry, double headed arrows in reaction mechanisms indicate the chain reaction showing how electrons move through the system. An oligonucleotide whose backbone uses backbones that has these elements would be optimal.

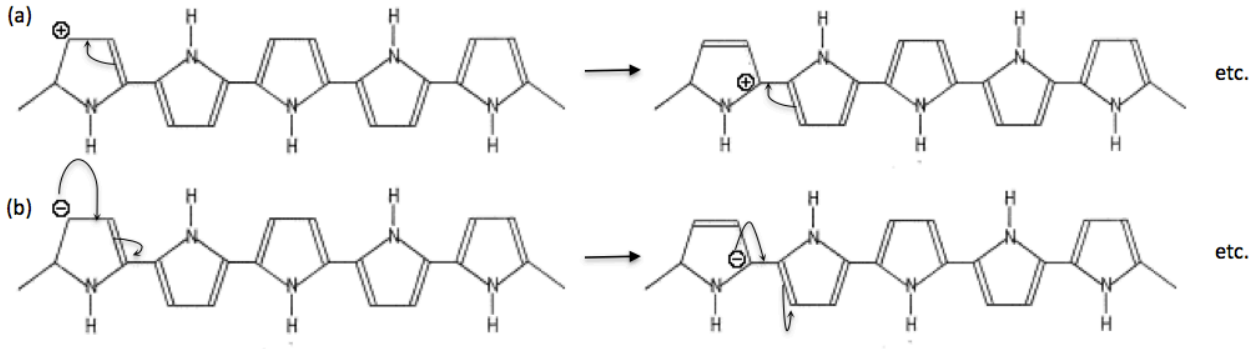
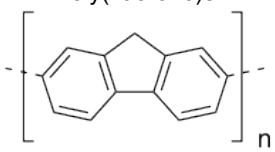
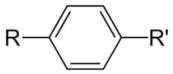
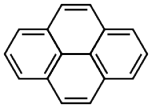
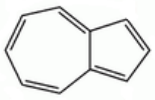
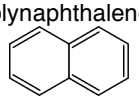
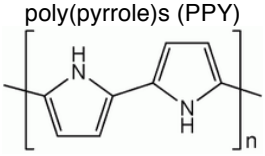
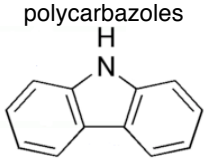
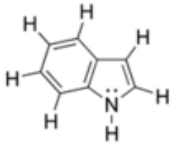
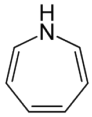
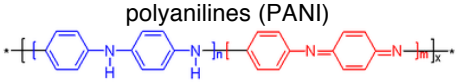
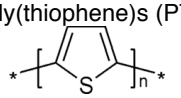
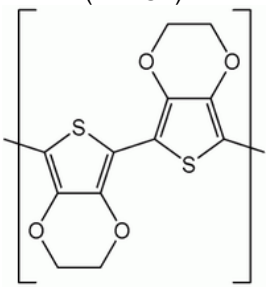
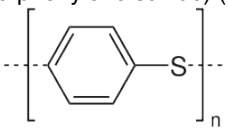
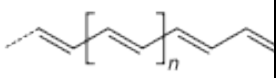
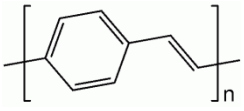


Figure 50: Mechanism showing how (a) holes and (b) electrons move through polypyrrole.

Table 1: Classification of conductive polymers. Names of the polymers are given above their structures.

The main chain contains	Heteroatoms present		
	No heteroatom	Nitrogen-containing	Sulfur-containing
Aromatic cycles:	Poly(fluorene)s  polyphenylenes  polypyrenes  polyazulenes  polynaphthalenes 	**The N is in the aromatic cycle**: poly(pyrrole)s (PPY)  polycarbazoles  polyindoles  polyazepines  **The N is outside the aromatic cycle**: polyanilines (PANI) 	**The S is in the aromatic cycle**: poly(thiophene)s (PT)  poly(3,4-ethylenedioxythiophene) (PEDOT)  **The S is outside the aromatic cycle**: poly(p-phenylene sulfide) (PPS) 
Double bonds :	Poly(acetylene)s (PAC) 	(Nitrogen has one extra lone pair)	(Sulfur has two extra lone pairs)
Aromatic cycles and double bonds	Poly(p-phenylene vinylene) (PPV) 		

To achieve higher mobilities, a family of redox reactions per polymer improves the conductivity of a material. For example, the study of reactions to modify polypyrrole will be different from the findings they discover for a different molecule. When described to electrical

engineers, the redox reactions are commonly compared to doping to electrical engineering. Usually, oxidation reactions are used to give “p-type” materials, but, in principle, reductive reactions should result in “n-type” materials, too. The existence of heteroatoms in polymers makes them in effect, “self-doped” and induces the cascade of electrons even more. Electron donating and electron withdrawing groups can be used to further tailor the HOMO and LUMO levels of these molecules.

Perhaps the biggest setback of conductive polymer technologies is in its mobilities compared to those of metals and materials in silicon-based technology. Undoped conductive polymers typically have very low “intrinsic” conductivities, on the order of 10^{-10} to 10^{-8} S/cm. Doping until saturation can lead to conductivities from 0.1-10 kS/cm. Compared to the conductivity of copper (596 kS/cm), and doped silicon (~0.1 kS/cm), this is not that bad actually.

An example of a high mobility organic thin film is made using P3HT, or poly(3-hexylthiophene), which is a type of polythiophene (see **Table 1**). This thin film’s mobility depends on molecular weight (MW) even after substantial morphological modifications of the film. Most research aims to make very crystalline polymers to increase mobility. Contrary to that research, high-mobility, high-MW films have a *less ordered*, isotropic, nodule structure, according to this paper [88]. And they determined that, consequently, the amount of in-plane π-stacking is not the primary cause of the dependence of mobility on MW. Although the results hold promise, this paper happens to be relating nano-scale phenomena to macroscopic definition of mobility. On the other hand, we are actually interested in just the nano-scale definition of electron transport and mobility.

The concepts behind conductive polymers can be applied in multiple ways to benefit nanoelectronics. As we have been alluding all along, using these ideas to make new oligonucleotides with conductive backbones may be one option. If we made an oligonucleotide with a backbone that has all the features of conductive polymers, we must consider how we can integrate it with a DNA nano-scaffold. Perhaps, before assembling the nano-scaffold, we could exchange out part of one of the legs and replace it with the new oligonucleotide. To be able to do this while keeping the other legs intact, would give the same effect as metallizing the aforementioned sections. To do this, the new oligonucleotide needs to have the same periodicity as DNA and it needs to use the same base pairs: in other words, it must be a complete DNA analogue. A completely different option is engineering a more conductive base pair that can be used in DNA. Di Felice’s work is based on the fact that guanine is the most conductive of the 4 classic bases. But, including uracil, which is used only in RNA, there are more synthetic bases that might conduct even better [89]. But none of the new ones have been

investigated for conductivity in DNA and enzymes may have trouble interacting with them in the major groove.

A number of technical issues limit the applicability of conductive polymers. Self-assembled monolayers are commonly used to measure charge-transport through new conductive polymers [90]. Such reliance on non-biological self-assembly means this field faces many of the same technical problems associated with molecular electronics, mentioned in Chapter 2, Section C. These include issues like ensuring electronic connection between organic and inorganic components of a circuit. The low solubility of most polymers presents challenges. Despite intensive research, much research correlating the relationship between morphology, chain structure and conductivity is yet to be done for many of these materials.

All in all, there is ample room for improvement with conductive polymers. According to one study, if the entire charge carrier density could participate, then the conductivity could surpass that of copper [91]. Other than that, this direction of future research could also benefit from an improved theory of carrier mobility in organic molecules.

C. Discussion

Nonetheless, there are many loose ends to the above proposal and many unanswered questions, waiting for the right speculative answers. This idea may show how biological self-assembly can be integrated to achieve goals of nanoelectronics, but in a way it is a "same-only-smaller" version of a transistor. And because the physics at the quantum level changes so drastically, conduction might not work as expected in this case. Nonetheless, this thesis has, hopefully, impressed upon the reader the importance of biological self-assembly and how it enables the addition of complexity.

There are quite a few different approaches this goal--for the sake of simplicity, we classify them into 3 umbrella categories:

(1) people working in "nanosynthesis" do the materials science legwork behind the nano-elements that could make future devices (the things we need to position or grow accurately on the nano-scale),
(2) device engineers are concerned with designing devices that work properly, and
(3) DNA organizers delving in the chemistry of the biological self-assembly behind making nano-scaffolds of different geometries.

Our solution would bring together what all three categories of people know, using biological self-assembly. Each of these research fronts probably has its criticisms about ideas like this one, and these are all issues that must eventually be addressed. In the following subsection, I would

like to touch on what these criticisms might possibly be. And going a step further, I hope to convey how, despite the importance and legitimacy of their concerns, biological self-assembly is still necessary to make computers out of laboratory-scale devices.

Possible Criticisms & Remembering the Higher Level Goal

Researchers and developers working in **nanosynthesis** know how to make quantum dots, quantum wires, carbon nanotubes, graphene sheets, and much more. They justify their interest in these metallo-inorganic compounds and nano-elements with their nano-electronic promise. But as Chapter 2 showed, although their findings are integral to nanoelectronic progress, if the products of their research cannot reliably self-assemble, they will only be "semi-solutions". To truly promote the transition from chemistry to circuitry in nanoelectronics, this field should try to veer away from depending on "anti-complexity" technologies.

So while M-DNA may not even be confirmed as a conductor, fit for nanoelectronic "consumption", DNA does have characteristics that many nano-objects do not. DNA has: (1) a family of well-characterized enzymes and proteins that can be used to accurately manipulate it on the nano-scale, (2) the ability to make a scaffold out of it, and (3) the ability to program location into an object by functionalizing it with DNA sticky ends. These are the successes of DNA: it is the champion of biological self-assembly. If the nanosynthesis people could find a material that can do these things, with that degree of accuracy, then that route should also be explored, too, for the purpose of nanoelectronics.

Device physicists and engineers are concerned with making, testing, characterizing, and understanding the transport within different, possible transistors, or other devices. Practically, a real transistor needs to have a couple of characteristics in its I-V curve that many of these laboratory scale devices do not have. For a single device, the physical limit for subthreshold swing is S = 60 mV/dec. There are circuit-level ways of achieving a subthreshold swing less than this but there must be something wrong with any *device* measuring S < 60 mV/dec. Once the ratio for dynamic energy to leakage energy is fixed, the optimal I_{ON}/I_{OFF} ratio is proportional to the logic depth divided by the activity of the circuit.[92] But to even begin talking about these circuit-level requirements of a MOSFET replacement device, we need to be able to construct circuits.

From a fabrication standpoint, materials like carbon nanotubes, nanowires, and graphene, need separate techniques to organize them into a working circuit. Even with the M-DNA transistor scheme, there are still some troubles of complexity that are left unaddressed. From a device physics standpoint, contacts to the source and drain of any nano-device present a problem. As contacts, small nano-scale leads would not provide enough energy states, to

function as a contact. More problems would arise from determining how we should treat such a complex real-world situation, in terms of physics. For example, how do you treat a quantum point contact in the Landauer Formula? More research on the experimental side, based on singular devices and arrays of devices could address these concerns. Nonetheless, there have been no other suggestions about how to add complexity on the large-scale for nano-element-based devices or nucleic acid-based devices. This emphasizes how integrating biological self-assembly is important, to even consider these circuit-level concerns.

DNA organizers focus on the chemistry of self-assembly, and often are not as concerned with the electronics or electrical properties of the materials they specialize in. Future research by people in this field may be more interested in DNA Repair proteins because these proteins might help manipulate metalized DNA (post-metallization). How might ligation occur on M-DNA? How would the base-flipping proteins work on M-DNA? While these concerns are important, this research must coordinate with efforts made by device engineers and nanosynthesis people to appropriately impact nanoelectronics. By employing their knowledge for the sake of nanoelectronics, researchers and developers in these three categories could achieve bigger and better things. Together, these efforts could revolutionize a field.

Getting Inspired by Nature

Admittedly, an electrical engineer's definition of electronic functionality is a concept more or less foreign to biology. A few metals, like magnesium and iron, are very common in organisms. Magnesium is needed for more than 300 biochemical reactions in the body. Blood needs iron to bind oxygen. So while none of these metal atoms exist there for the purpose of conductivity, biochemical studies surrounding them tell us about how organic materials interact with metals on the nano-scale. For the most part, the type of conductivity that matters in biology is the conduction of ions: either in across a cell membrane, or in the brain. Nonetheless, biological chemistry offers ways of controlling conductivity, by means of molecular interactions.

Certainly, biology would be the source of self-assembly's penultimate example. When a biologist thinks of "self-assembly", chances are they imagine microtubules (spindle fibers). Tubulin monomers polymerize to form microtubules: it is similar to the self-assembly of other super-molecular structures like virus capsids [93]. There are multiple environmental concentrations that can cue the formation of microtubules, even parameters that reflect the polymer level, like turbidity or viscosity. For each of these parameters, formation begins with a lag phase where no microtubules form, followed by a phase of exponential growth, and a stable plateau phase. At low polymer concentrations, no microtubules are formed. Above a critical concentration, microtubules increase proportionally with total tubulin concentration, behaving as

if in equilibrium with the pool of unassembled tubulin. In biology, the mechanism of nucleation is complicated, and is usually initiated by centrosomes. The mechanism for how depolymerization occurs is related to how this process is chemically integrated with the hydrolysis of guanosine-5'-triphosphate (GTP) to guanosine diphosphate (GDP). The study of the self-assembly of such biological elements may provide hints of how to reach the pinnacle of "self-assembly" for nanoelectronics. Perhaps, to do so, we must think through the chemistry much more carefully, and not simply assume and borrow everything from silicon-based technology.

It may be hard to imagine that examples of nature doing electronic things at greater efficiencies even exist. But when the complaint of organic electronics is that conductivities are so low, electrical conduction in electrical eels, and other organisms that use electrocytes, definitely seems interesting. For hunting and self-defense, the electric eel can generate powerful electric shocks of up to 500-600 V, and a full ampere of current (500-600 W). This amount of energy can be fatal for even a human. Three organs are responsible for making this amount of electrical energy: the main organ, the Hunter's organ, and the Sach's organ. The Sach's organ contains thousands of electrocytes, each of which can produce 0.15 V: when discharged together, this creates the high voltage, which is used in nature to stun prey. The electric eel uses sodium ions to deliver these shocks. In fact, electric rays and electric catfish make use of ionic conduction (not electron conduction), so this is another point that might be worth reconsideration in this engineering problem. Do we want to stick to electron conduction, or might ionic conduction be able to achieve the same ends?

The more discoveries branch synthetic biology to electronics, the closer we are to using more than just biological self-assembly for the sake of nanoelectronics. A pertinent question for this turn of research would be: how close are we to inventing an electronic organism? Organisms are entities that have the ability to replicate amongst themselves, each of which have an internal metabolism, and adapt to changing environments [[70]]. If all the different levels of organization that occur in a multicelled organism could be replicated to make an organism with electrical functionality, this would be very interesting. Today, synthetic biology uses cells as factories to make certain proteins or molecules, on a very regular basis. Eventually, using the same kind of thinking to make an electronic organism could lead to a large-scale circuit that is not unlike an organism. A number of enzymes, compartments, organelles, and metabolic processes could achieve different electronic ends. The fact that the electrical engineer's concept of electronic functionality is completely foreign to biology makes it even more challenging to bridge the gap. So we are still very far from making a completely electronic organism. Perhaps a more plausible question could be: how far can one go *in vitro*, with using the information DNA

sequences encode for, to realize non-biological functionality? And thinking along these lines helps advance the interface between biology and electrical engineering.

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There is a shift towards making computers more parallel, but incomplete efforts have lead to various issues with modern day computers. So, why stop at the device level? In the name of reformation, why not re-evaluate even von Neumann architecture? The von Neumann bottleneck is developing because program memory and data memory cannot be accessed at the same time. Basically, the CPU has to wait for data to transfer, because although CPU speed and memory has increased, the throughput has not. Memristors and related devices aim to address this problem. A different problem with computers today is the use of multi-core processors. Without programs that efficiently use all the cores in a computer, this kind of computer augmentation is rendered useless. So why not build a device that belongs in an architecture that is easy to program things that require parallel computation? A different problem plagues the fabrication and VLSI side of computers. As devices increase in complexity, defect and contamination control become even more important. Because defect tolerance is very low in present systems, every transistor has to work perfectly, and when 100s of millions of transistors populate each chip, that becomes a huge challenge. So why not build a device that can deal with these kinds of defects, the way the body does?

So do we really want something that functions like a MOSFET? How does the brain work? Nature contains other possible units for information processing: the brain, for example, is made up of neurons connected by synapses. A synapse is a connection between two neurons that allows information to travel from one to the other. If we really wanted to make the greater system function like the brain, the unitary device could function like a synapse. The brain relies on ion conduction, which we have not considered for our case. Depending on how active a synapse is, the strength of that connection changes: this is said to give the brain the learning and memory capabilities. The brain's plasticity refers to its ability to constantly grow, change, adapt and remap itself over the course of a lifetime. We are interested in the brain because, in a sense, it is the culmination of evolution, in functional computing.

Consider a chip that can mimic the plasticity of the brain: in particular, how the brain's neurons adapt in response to new information. This silicon chip, built in 2011, can simulate the activity of a single brain synapse using about 400 transistors [94]. The transistors used are analog, with subtle graduations that act more like ion channels. But why build a silicon-based circuit that acts like a neuron, when we have the option of creating a single device that could behave in the same way? The NOMFET is one such nano-device that functions like a synapse

[95]. But such a device probably can only do so much to mimic the plasticity of a brain. Neuromorphic computing is the name of this field concerned with brain-inspired computing.

As far as chemistry goes, the human body is a miracle in its own right, which is ultimately a web of biochemical pathways. When one biochemical pathway does not work, due to the lack of molecular resources, or when certain enzymes are not made in sufficient quantities, the body finds alternate routes to make do. Imagine, if a computer could be made such that even if defective devices are used, the computer can work around these things, and still be useful?

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When we set off to help the delay the end of Moore's Law, and illustrated the usefulness of biological self-assembly to the cause of nanoelectronics, we showed how necessity is the mother of invention. We invent things when we need them to work differently. But perhaps, nearing the end of this discussion, it may be appropriate to notice that invention, too, is the mother of some necessities. We proposed a way to position and make nano-elements accurately, and control their conductivity. And if this "invention" works for its purpose, although we would have solved a problem, this last chapter has illustrated how this "invention" leads to the need to be able to make RecA and M-DNA processes compatible. And find ways to coordinate change with the formation of M-DNA in the tensegrity-triangle based nanoscaffold. So it seems like even invention is the mother of necessity.

Either way, this thesis stands as a suggestion for the sake of the progress of nanoelectronics. And if continued imagination, engineering, and cost allow, many possibilities that seemed impractical before might be within the grasp of reality.

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Picture references:

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- Polypyrrole (http://www.sciencedirect.com/science/article/pii/S0956566301003128)

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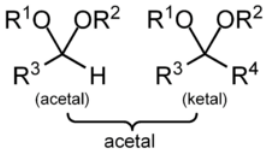
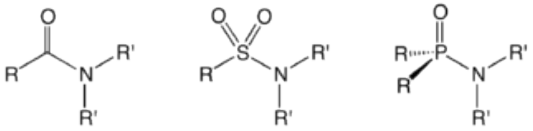
GLOSSARY OF TERMS AND ABBREVIATIONS

#-mer - polymer with # monomers linked together
A - adenine
A-DNA - another type of DNA with a slightly different geometry than typical DNA. The twist is still right-handed, but the major and minor grooves have different sizes. (see the diagram at Z-DNA)
acetal - a functional group consisting of a carbon atom attached to two oxygen atoms via single bonds. If the fourth constituent is a hydrogen, it is called an "acetal" and if the fourth one is another alkyl group, it is called a "ketal": but, both ketals and acetals can be referred to as acetals.



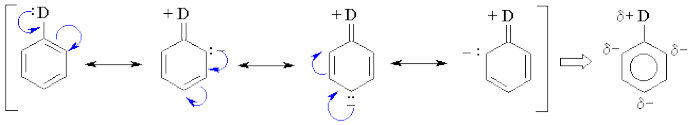
alkyl - a functional group consisting of any structure containing only carbon and hydrogen atoms.
amide - a functional group where two alkyl groups are attached to a nitrogen, whose third constituent is an oxygen, sulfur, or phosphorous group that is involved in a double bond.



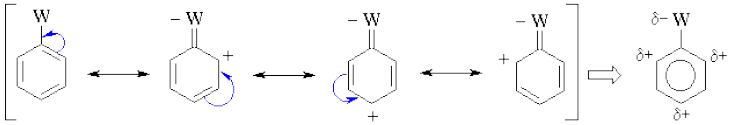
amine - a functional group that contains a nitrogen connected to 1-3 alkyl groups.
amplify - increasing the concentration of a DNA strand in solution.
anticomplexity technologies - technologies used for nano-manipulation that have low throughput and high cost. These include AFM, electron beam lithography, and more. Dependence on microscope technologies, like SEM and STM, also implies that these cannot be automated in anyway and that self-assembly is not natural.
aromatic ring - a carbon ring with alternating double and sing bonds between the carbons. There are 4 rules that must be fulfilled to satisfy the definition of aromaticity. The system must have/contain:

1. a delocalized conjugated π system
2. a coplanar structure, with all the contributing atoms in the same plane. This usually implies sp^2 hybridization.
3. Contributing atoms arranged in one or more rings
4. A number of π delocalized electrons that is even, but not a multiple of 4, in other words 4n + 2 electrons, where n = 0, 1, 2, 3, … (this is known as Hückel's Rule)

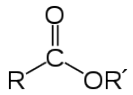
asymmetric ligand field - a way to summarize the physical placement of the atoms and bonds around the metal ions along the center of M-DNA
B-DNA - biological deoxyribonucleic acid. This is the same as classic, original DNA. (see the diagram at Z-DNA)
bp - base pairs: refers to Watson-Crick pairing.
C - cytosine
cation - positively charged ion
cleave - in chemistry, this refers to the breaking of a chemical bond in a molecule to give smaller molecules or radicals. So with respect to DNA, this refers to cutting DNA.
CNT - carbon nanotube
conjugated pi system - a molecular system of connected p-orbitals with delocalized electrons. This usually is in the form of alternating single and double bonds, which in general, lowers the overall energy of the molecule and increases stability
denaturation temperature - in DNA, oligonucleotide, and protein chemistry, this represents the temperature where the concentrations of the folded and unfolded states are in equilibrium. An indicator of how strong the bonding in the molecule is, we can assume the molecule completely unfolds at any temperature above this one.
denature - in DNA and oligonucleotide, this verb implies the breakage of all the hydrogen bonds between the Watson-Crick base pairs.
DNA - deoxyribonucleic acid
DoS - density of states
dsDNA - double-stranded deoxyribonucleic acid
electron donating group - a functional group which releases or contributes electrons to a reaction center or aromatic ring



electron withdrawing group - a functional group which draws electrons away from a reaction center or aromatic ring



ester - a functional group RCOOR, where the carbon could be replaced by a sulfur or even a phosphorous atom.



ethyl - name for a $–CH_2CH_3$ group, a type of chemical functional group, specifically, a type of alkyl group.
FET - field-effect transistor.
functional group - a specific group of atoms that form a "submolecule" so to say. These are motifs in organic chemistry. For example, –OH group is called "hydroxyl-".

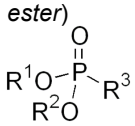
G - guanine
HOMO - highest occupied molecular orbital
homologous - where bp sequences are similar between two strands of DNA
homologous recombination - a type of genetic recombination in which nucleotide sequences are exchanged between two similar or identical molecules of DNA. In particular, this is where ssDNA forms a triplex with dsDNA: RecA facilitates the formation of this structure.
hydrogen acceptors - the molecule containing the electronegative atom involved in a hydrogen bond
hydrogen donors - the molecule containing the hydrogen atom involved in a hydrogen bond
hydroxyl - name for an –OH group, a type of chemical functional group
in vitro - in test tube conditions
in vivo - in a living organism
ionization potential - energy required to remove electrons from gaseous atoms or ions. In molecules, it refers to the difference between the energies of the neutral molecule and its positive ion
K_a - affinity constant, measure of the strength of binding of the components in a complex. For components A and B, and the binding equilibrium A + B ⇆ AB, the association constant is given by:

$$K_a = \frac{[AB]}{[A][B]}$$

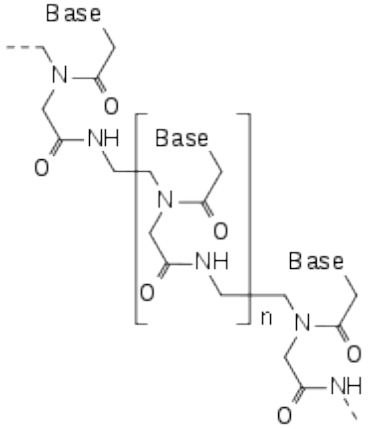
The larger this value, the tighter the bonding.
K_d - dissociation constant, measure of the tendency of a complex to dissociate. For components A and B, and the binding equilibrium A + B ⇆ AB, the dissociation constant is given by:

$$K_d = \frac{[A][B]}{[AB]}$$

The smaller this value, the tighter the bonding. This is the reciprocal of K_a
ligand - a smaller molecule or ion that is able to bind with a larger molecular
ligate - to bind chemically. This typically refers to attaching two DNA strands together.
lone pair - a valence pair of electrons associated with an atom but not participating in ionic or covalent bonding.
LUMO - lowest unoccupied molecular orbital
M-DNA - metalized DNA
M-PNA - metalized PNA
methyl - name for a $–CH_3$ group, a type of chemical functional group
MW-CNT - multi-walled carbon nanotube
nucleobase - a type of nucleotide that contains a particular base: either A, C, G, or T. *See nucleotide.*
nucleotide - the "unit" of ssDNA, which consists of a sugar, phosphate group, and a base.
NW - nanowire(s)
oligonulceotide - a short polymer of two to twenty nucleotides (aka oligo)
PCR - polymerase chain reaction
phosphonate - organic compounds containing /-C-$PO(OH)_2$ or /-C-$PO(OR)_2$ groups. They can be classified as general esters of phosphonic acid (*see ester*)

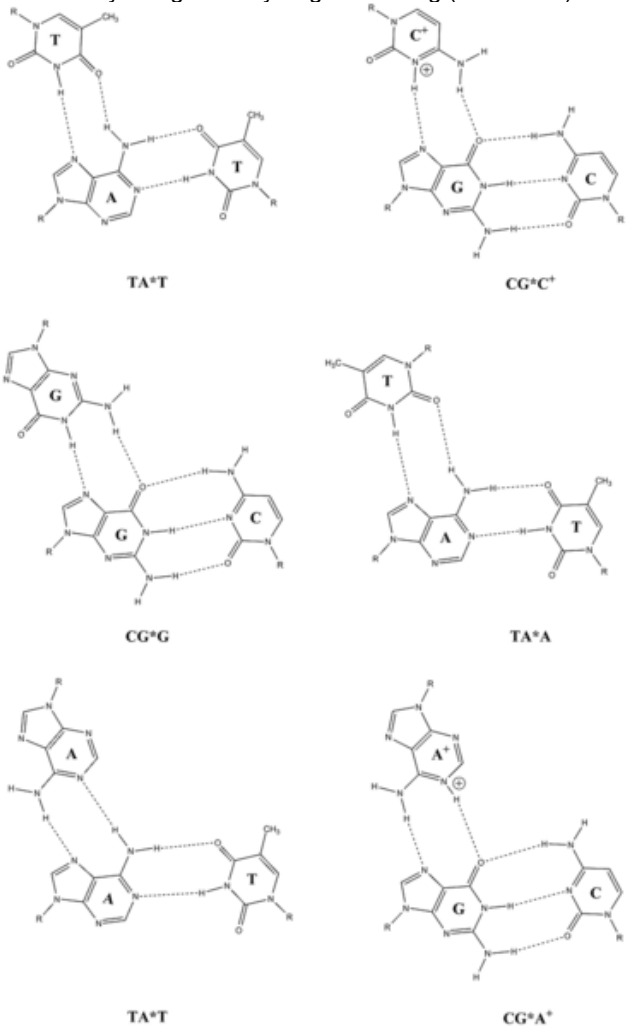


phosphoramidate - $(RO)_2PNR_2$ is a monoamide of a phosphate diester often used in the chemical synthesis of DNA, RNA, and other nucleic acids and their analogs (*see ester*)
PNA - peptide nucleic acid. Although it has the same periodicity as DNA, with a peptide-like backbone instead of the phosphodiester DNA backbone, making it completely neutral

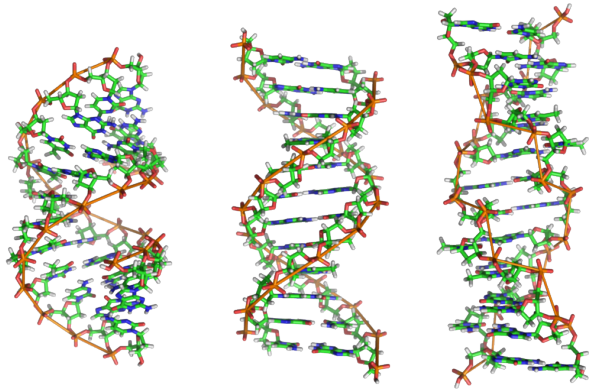


pol - polymerase
poly(*dA*)-poly(*dT*) DNA - dsDNA that only has the adenine base down one side and thymine down the other side.
poly(*dG*)-poly(*dC*) DNA - dsDNA that only has the guanine base down one side and cytosine down the other side.
poly(*dG-dC*) DNA - DNA that only has the guanine base. If it is single stranded, then guanine is the only base present, and if it is double stranded, cytosine is present as well.
programmable matter - any matter that inherently has the ability to perform information processing (i.e. DNA)
RecA - protein essential for the repair and maintenance of DNA (its from *E. coli*)
RNA - ribonucleic acid. This is different from DNA in that the sugar HAS the hydroxyl group and the
ssDNA - single-stranded deoxyribonucleic acid
sticky ends - referring to the properties of the end of a molecule of DNA or a recombinant DNA molecule. If the lengths of the two strands of DNA are different at the end, then the lonely bases can form hydrogen bonding with complementary sticky ends. The bondage is temporary until ligase forms a covalent bond between the sugar-phosphate groups of the respective backbones. (*see ligase*)
SWNT - single-walled carbon nanotube
T - thymine
thiol - name for a –SH group, a type of chemical functional group

triplex - triple-stranded structure of DNA that is enabled by Hoogsteen hydrogen bonding (see below)



Z-DNA - another type of DNA with a slightly different geometry. The twist is left-handed, and the major and minor grooves are of different size. (see image below)



A-DNA B-DNA Z-DNA

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