Seth Fichtelberg

Last summer, I spent five out of seven days a week at Brookhaven National Laboratory's, splitting my time between the lobby of the Education Building and a laboratory in the Center for Functional Nanomaterials. It's really not as bad as it sounds; I enjoyed myself thoroughly because I was involved in a field that I found incredibly interesting. In fact, it was a very unique experience in which I'm glad that I took part.

Looking back, I hadn't exactly pictured myself working there. At the end of my eighth grade school year, some representatives from the high school "Independent Science Research Program" came to visit my class. They gave a short presentation to my science class about their program, emphasizing that it was for an elite group of motivated students committed to furthering their interest in the field. For whatever reason, I decided that I was not going to apply. I recently got to go back to that same classroom, this time as a member of that program, and present the program to middle school students as my predecessors had before me. I think I now understand why I was hesitant to submit an application. It's intimidating to young students, especially in a middle school setting, to see older students give presentations about how inconceivably hard they worked on their incredibly erudite projects. In addition, no one wanted to be the first student to take an application in front of the class and thus become the subject of ridicule by his/her peers.

Enough excuses, however: the simple fact is that I should have applied before entering high school. I squandered my freshman year, not wanting to overload myself while still becoming accustomed to the new environment. As luck would have it, however, I was approached midway through my sophomore year by members of the research club. They were on a recruitment drive because the program was understaffed.

Having aged two years (and apparently having knocked some sense into my head), I jumped at the opportunity to join. That fateful moment was the first step on my journey to my Intel project.

I was quickly accepted into the research program, and soon after, I started reading scientific journals as a part of my new weekly assignment. I began systematically leafing through publications, looking for something I might be interested in. It was in one of these science journals that I first came across a practical use of nanotechnology. I distinctly remember reading an article about a nano-sized cutting device created from carbon nanotubes; it was to be used to cut the organelles of a cell so that scientists might discover what lies inside. I was instantly attracted to the idea of an infinitesimally small knife made from synthetic materials. Today, I've thought it over, and I imagine that my curiosity stemmed from my experiences as a child. For one of my earlier birthdays, my parents had gotten me a low power microscope, and ever since I've been captivated by everything that is invisible to the naked eye. At any rate, I dog-eared the article and presented it to my advisor as an example of my interests. She discussed the article with me, quietly tucked it away in my folder, and asked me to find other articles.

The next time I saw that article was almost exactly a year later, in my junior year of high school. Traditionally, during the summer of their junior year, students in the research program go to a laboratory or university to do higher level research, in hopes of competing in Intel, among other competitions. My advisor pulled out my folder in order to refresh her memory, in hopes of finding a clue as to where I should be placed over the summer. The piece about the carbon nanotubes floated out when she opened the folder, and she immediately had an idea. Apparently, a new building had been opened at

Brookhaven National Laboratory that dealt specifically in nanotechnology. My advisor had a friend at the lab and offered to make a call, provided I was interested. I don't recall my exact response, but I'm certain it was a mix of shock and sheer delight. I was absolutely certain that I had found where I wanted to be placed. A few days and several phone calls later, I had a mentor, a lab, and a proposed experiment, and I was cleared by security to enter the Brookhaven campus.

I met my mentor on the first day of work, after the jarring 45-minute bus ride on the infamous Long Island Expressway. He introduced himself as Scott Bronson, and he is the man to whom I dedicate my work and to whom I attribute my success. He has always been tremendously helpful and supportive, even in those crucial, clumsy moments after I got off the bus. He led me to his building, the Office of Educational Pursuits. I was slightly confused, to say the least; I thought I would be spending most of my time in the lab at the Center for Functional Nanomaterials. It turns out that the life of a young intern is anything but glamorous, as I spent most of my time sitting in the lobby with an Apple on my lap, unable to move because I was connected to the wall socket. This is where I did a large portion of my project: here, in the air-conditioned expanse overlooking the rear parking lot. While many of the other interns were in the lab, I sat in a comfortable armchair in a climate-controlled environment; I like to say I got the better deal. I dealt mainly in computer modeling and computer science, most of which I had to teach myself, and so this environment was fitting. If I had one suggestion for other high school students seeking to perform experiments at the Intel level, it is to make the best of what you're given. I will confess that at first, I was disappointed not to be doing work in a clean room and wearing a lab coat, but I came to enjoy plugging away at my computer (I'll get into

detail about what I was actually doing later). I'm certain that learning to accept my fate, and making modifications to find amusement in my work is the only thing that kept me sane during the 40 hour weeks I had performing a repetitive but thought intensive task. I certainly don't intend to discourage anyone from pursuing an internship in a lab, but it is very cerebral and rigorous work. It takes focus, drive, and initiative to take advantage of such an internship, but if a student has those traits, then it will be hard for him/her *not* to succeed.

So far I've been speaking of all the difficulties of my internship, and overlooking all the positive aspects. It's true that I did a fair amount of work during the summer, but I also enjoyed some of the unique opportunities presented to me at Brookhaven. Because I worked at a nationally recognized and federally funded laboratory, I was treated to interesting guest lectures on a near weekly basis. Brookhaven is the site of RHIC, a famous particle collider, so many of these presentations were on particle physics, which is incredibly interesting to me, though perhaps not the average person. I remember that at one point during my time on campus, I even had a videoconference with an NYU professor who was working at the LHC, another collider made famous by Dan Brown's Angels and Demons. The work that I did on my project, along with these incredible seminars, helped portray science in a new light for me. No longer did "science" connote 8th grade biology labs and cheap chemistry tricks that usually ended in explosions; now I had been exposed to real experimentation, which meant complex topics discussed over freshly brewed coffee in small concrete rooms with swivel chairs. It wasn't pretty (science never is), but I loved it.

Research Section

Modeling and Sequencing the Elements of a Bent Linear DNA Array

Nanostructures are constructed from carbon nanotubes as well as small nanoparticles on the scale of 10^{-9} meters. In recent years, DNA has come to be used in nanotechnology as a structural base material. DNA is prized in this regard for its unique property of Watson-Crick complementary base pairing. This natural process can be exploited to allow for the self-assembly of segments of DNA. Base pairing itself is an extremely primitive example of self-assembly. Because DNA naturally uses this process to form many different shapes, it is a very pliable material that is easily shaped simply by altering nucleotide sequences. Thus, self-assembly of DNA is cheaper, easier, and more desirable than physical manipulation.

There has been much research done exploiting base pairing as a method to synthesize 2D or even 3D shapes, known as arrays. In 2005, research was done using gold nanoparticles chemically fastened to DNA segments. The DNA segments were then allowed self-assemble into arrays of conductive materials. The result was a nano-sized conductive strip consisting of gold nanoparticles that could be used to further decrease the size of microprocessors in computers, as well as for a number of other functions. It was speculated that DNA could be used for similar purposes in the fields of microbiology and chemistry, particularly pharmaceuticals.

The official title of my project was *Modeling and Sequencing the Elements of a Bent Linear DNA Array*. Roughly translated into English, this entails the creation of a structural model of DNA and the generation of a DNA sequence that, when allowed to self-assemble, creates this shape. I was given a tremendous amount of autonomy over the

process: I chose the shape, made the model, and then designed the sequence largely by myself.

Though the general plan of what I actually accomplished is easy to follow, the details are significantly less so. The premise of this project was actually based on another study, done in 2006. As I learned, most experiments are in some way a derivation of a previous experiment, which contributes to our notion of science as a slow progression. This concept is contrary to popular belief, which holds that most projects are breakthrough experiments on the fringes of the human understanding.

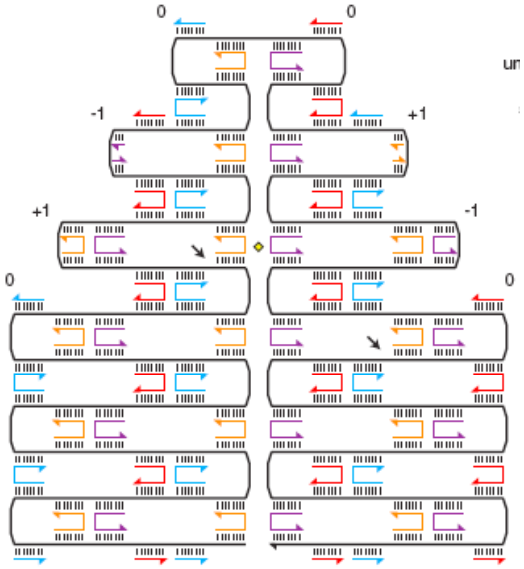

Figure 1. A Representation of the DNA Origami Experiment. The black strand illustrates the scaffold template strand.

Figure 1 illustrates the most famous of DNA nanostructures: DNA origami. This experiment introduced the concept of a single, continuous strand that runs throughout the entire structure and serves as a foundation for self-assembly, called the "scaffold" strand. Here, the black strand illustrates the scaffold strand, which serves as a template for the other strands that attach to it, illustrated in colors. To model the 2D tiled array in my experiment, DNA Origami was the basis for the concept and terminology of the continuous scaffold strand. That is, I borrowed the concept of the scaffold strand from

this experiment because it was a simple yet effective method of creating the 2D shape that I desired. Though the shape here was something similar to a pyramid, I would adapt the design in order to create a shape that I had chosen.

Deciding on the figure I would make was my next major hurdle. In the end, I decided on something simplistic, but with room for increasing complexity; I chose a zigzag pattern, which was easily repeated and which had enough freedom in its design in order to incorporate more complex elements when the time came. In order to create this pattern, and in order to test the limits of the self-assembly of the DNA, I had to conceptually break the array into three basic shapes. Two of these building blocks created an angular deflection, or bend, in the array to create the zigzag, while the third was a straight piece of DNA that just extended the array. The two pieces that altered the angle were modified versions of a common type of DNA junction: the Holliday Junction. Figure 2 (below) depicts the Holliday Junction, which is composed of four individual strands of DNA. Each spot where two different colored strands are joined constitutes an arm. There are four arms in a Holliday Junction. Figure 2(a) demonstrates that the angle formed between adjacent arms of different colors is 60°. Following the green strand, it is easy to see that it enters the junction and then turns on itself and is displaced 60° from the incoming strand upon exiting. This is more visible in Figure 2(b). The utility of the Holliday Junction lies in its ability to direct a strand by a specified and constant number of degrees. In my experiment, the Holliday Junction was used in conjunction with the scaffold strand concept from above in order to deflect the strand and create the zigzag shape. Figure 2(c) illustrates a modified Holliday Junction created using an innovative method devised for this experiment. By fitting the ends of the junction with a special

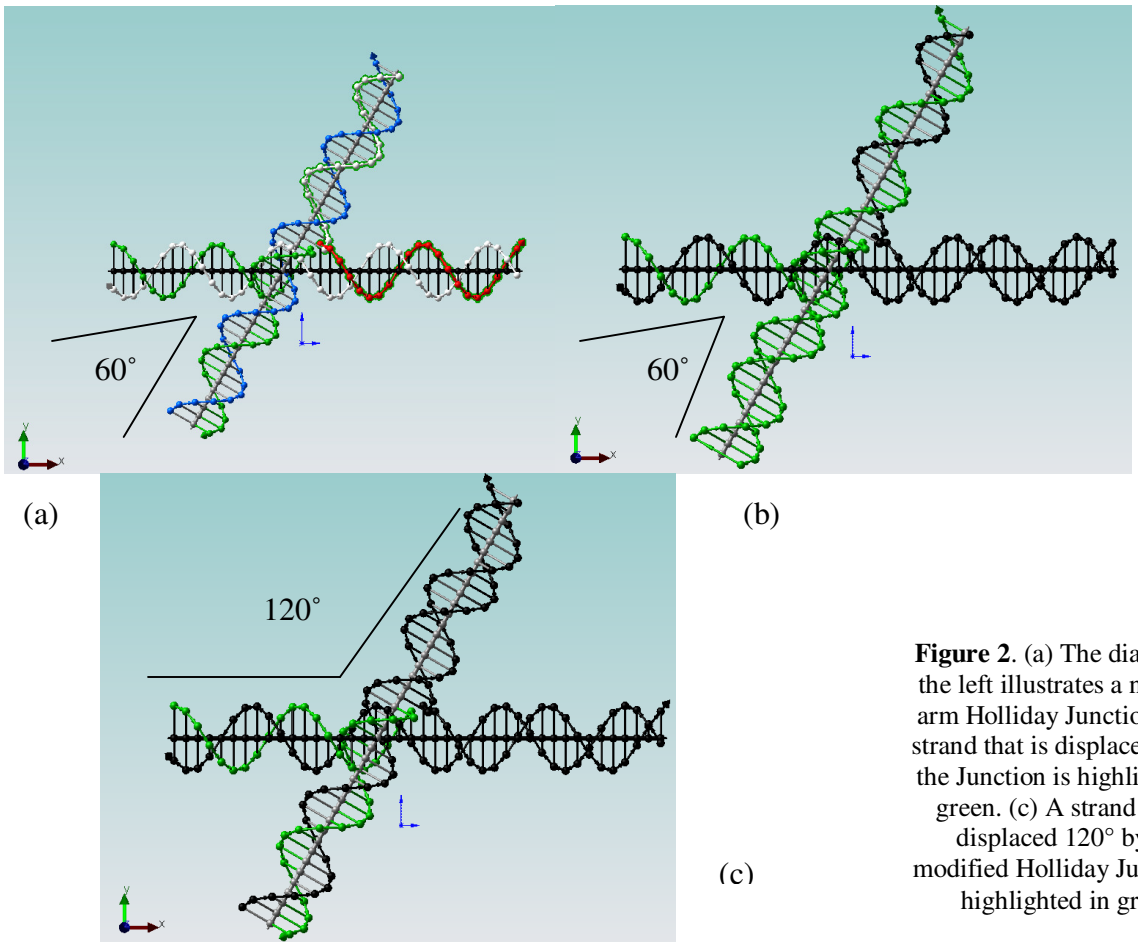

Figure 2. (a) The diagram on the left illustrates a normal 4 arm Holliday Junction. (b) A strand that is displaced 60° by the Junction is highlighted in green. (c) A strand that is displaced 120° by the modified Holliday Junction is highlighted in green.

sequence, it was possible to create a 120° deflection, instead one of 60°. With the creation of these structural models, I had almost all the tools I needed to begin sequencing.

However, one major obstacle remained; in projects such as mine, material costs often run high because the price of synthetic DNA is great. In order to have my theoretical array become economically feasible, I had to find a way to reduce the cost of obtaining multiple iterations of a repetitive section of DNA. The solution: Rolling Circle Amplification, a natural process of DNA replication. RCA is a method for the duplication of much longer arrays, and even replication of pre-made DNA structures. RCA is incredibly flexible and reduces material costs to a fraction of their original size, because

only one iteration of a sequence would be needed to reproduce thousands. Though the process is too complicated to explain in great detail, there are a few simple, key steps. First, the complement of the DNA that is to be replicated is formed into a circle. This is necessary because the next step involves the inclusion of an enzyme, polymerase. This enzyme travels around and duplicates the complement of the circle, thus creating an exact copy of the intended DNA strand. This process repeats ad infinitum, unless an outside force causes it to stop. Combining this technique with the ones discussed above, it becomes possible to create a structure by sequencing a simple foundation (the scaffold), replicating that with RCA, and adding the appropriate numbers of additional strands to complete the structure and deflect the scaffold by 60° or 120° at the designated sites. This was the basic theory of my project.

Now having devised all the tools necessary to synthesize my zigzag array, it was thus necessary to create the sequences that would self-assemble correctly. I had already made the physical models for the three necessary elements (see Figure 2 above for the results) and so all that was left was to impose nucleotides (A's, C's, T's, and G's) over these models. This process was done using Sequin, a command driven program designed to run in an archaic Unix environment. For this step of the project, it was necessary that I learn a small amount of programming and other computer science in the Unix environment so that I would be able to effectively operate the program. The Sequin program was rather simplistic; it merely generated random five base sequences according to specifications inputted by the user. Then the user selected appropriate sequences that the program had generated and added them manually to a model of the junction. It was up to me to add guidelines to dictate the generation, such as not to include certain repeat

sequences such as “GGGGG,” because these posed special hazards to the array. Sequencing the spacer strand, which was completely straight, was easy. The spacer was necessary to accommodate for the potential bonds between two adjacent Holliday Junctions. If the two structures drew too close to each other, it would be inevitable that they would come into contact. Should they do that, there would be a high probability that they would bond and effectively ruin the array. Thus, the spacer strand simply helped to avoid junction overlaps. Because it was straight and served such a limited function, it was sequenced quickly. Sequencing the other two elements, the two Holliday Junctions, was a bit more complex, because special commands were necessary in order to create particular features of the designs. All of the sequence generation was done utilizing a criton of five. In layman’s terms, this means that each five base sequence generated was unique among other five base sequences that had been generated. This was important to the next phase of my research: the verification stage.

It was necessary that the sequences be checked for compatibility. Incompatibility among sequences generally meant that there were two five base sequences that were exactly alike, which could cause problems when the structure was made in the lab. Thus, I had to use another special command in the Sequin software: the MISMATCH command. This utility allowed the program to check for such repeat sequences, and highlighted potential issues with what I had generated. It did not fix them however; that process was manual. After all the errors had been resolved, the sequences were deemed final, and one final round of models was created in Microsoft Excel that superimposed the sequences on the actual structures. These structures (below, in Figures 3 and 4) served

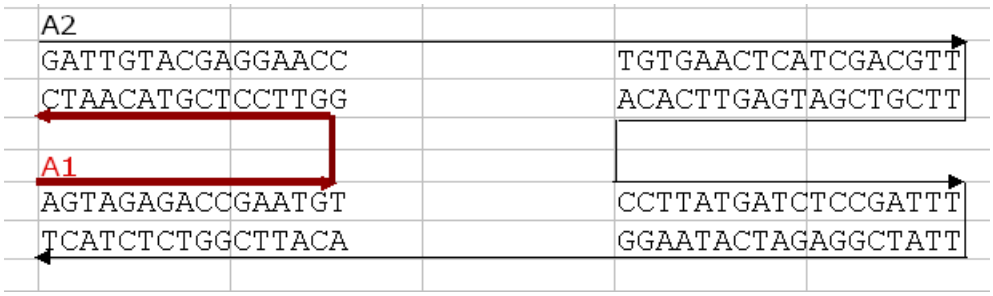

Figure 3. An Excel Model of the 60° Holliday Junction with Sequences

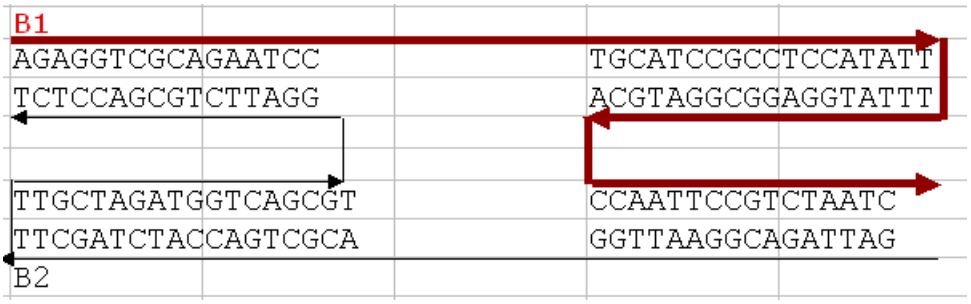

Figure 4. An Excel Model of the 120° Holliday Junction with Sequences

as a final precaution against error before the sequences were ordered from the scientific supply company. Because the models had verified the sequences and the structure, a grant was requested to synthesize the array. The Assistant Laboratory Director in the Department of Policy and Strategic Planning at Brookhaven National Laboratory allocated funds under the Directed Royalty Project 09035 to create an array using these elements in a lab setting. This grant was recently awarded and work has commenced upon the creation of the array. High power microscopes will then view the resulting array to verify that the structure present is the same as the intended structure. If that is true, then the experiment will be a success and the sequential and structural work will be validated.

Work in this field holds innumerable possibilities and applications. Scientists have recently exploited the process of DNA self-assembly to create a CDM, or Contractile DNA Machine. This machine is a nano-sized pair of tweezers that can be

used to manipulate nanoparticles and other similarly sized objects. This of course can be used to synthesize nanostructures from other materials, such as carbon nanotubes, using DNA nanotools. This experiment itself has potential use in the creation of other tiled arrays. The generation of sequences in this experiment was based around the premise of a zigzag shape produced by RCA. Because the strands were sequenced with this in mind, the overall architecture of the sequences was different than those sequenced individually. This adaptation of the DNA Origami experiment allows easier and cheaper synthesis of the same strands. Additionally, this experiment first documented the creation of a Holliday Junction with a 120° deflection. This experiment can thus be used as a technique to model and sequence elements of other self-assembly experiments in order to effortlessly and inexpensively synthesize a zigzag array for other purposes, such as in the CDM.

Finally, this experiment successfully created computer models and sequences of the elements required for a zigzag DNA array. These elements were effectively designed such that rolling circle amplification can be used to reproduce the necessary elements, allowing the creation of the array in the lab.