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At the March 5, 2010 UW-Madison Chemistry Department Colloquium, Prof. Bassam Z. Shakhashiri, the director of the Wisconsin Initiative for Science Literacy (WISL), encouraged all UW-Madison chemistry Ph.D. candidates to include a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

Over 50 Ph.D. degree recipients have successfully completed their theses and included such a chapter.

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Restructuring of Large, Double-Stranded DNA Molecules through Self-Assembly to form Microscale Objects

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of

Doctor of Philosophy
(Chemistry)

at the
UNIVERSITY OF WISCONSIN-MADISON
2018

Date of final oral examination: 12/11/2018

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Chapter 5: DNA Self-Assembly for the Non-Scientist

5.1 Introduction

Almost everyone has heard of DNA. It's generally thought of as the molecule that is passed down from parent to child, with half the DNA coming from one parent and half from the other parent to make a unique individual very similar to and very different from each parent. This is why most people think of DNA as *gene*tic material: because it is the physical object (the material) used to pass *genes* (the parts of the DNA that provide the instructions to change you from a single cell into a human being) from one generation to the next. Scientists such as myself are taking advantage of the properties that make DNA useful as genetic material to repurpose it as a building material. Using DNA, we can make a variety of intricate, molecular-level objects that have a many uses, from the development of new cancer treatments to novel microelectronics and molecular machines (see Figure 14, page 483). I have developed new ways of using DNA to create larger and potentially more complicated structures out of DNA. This opens up new avenues of exploration and should allow for additional useful materials to be formed out of DNA.

5.2 So What Can We Currently Make With DNA?

Scientists have been using DNA as a building material for decades, and currently the most popular technique for doing so is called DNA origami. This is an apt name for it because it really is folding a DNA molecule into assorted shapes much like traditional

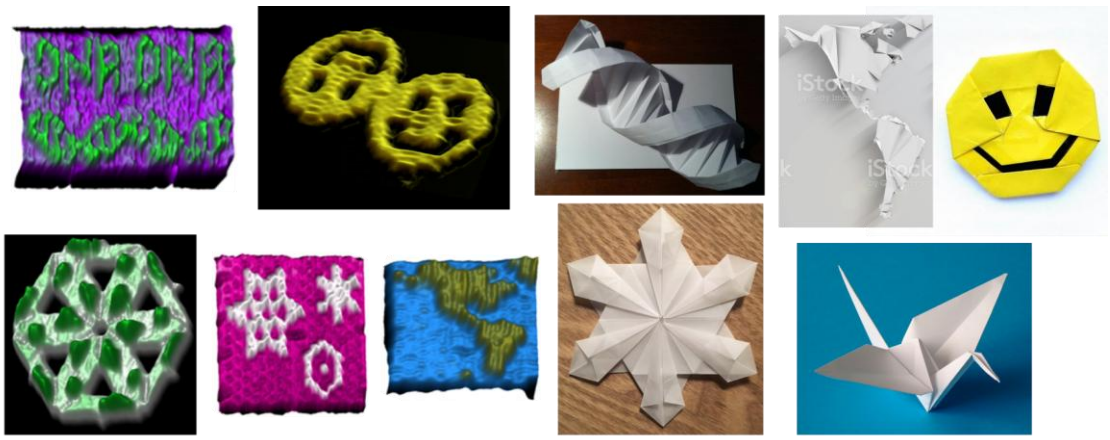

Figure 1. Example DNA origami[7] (left) and paper origami[8-11] (right) objects.

origami folds a piece of paper (Figure 1). However, while some tiny objects can be made with traditional paper origami, DNA origami—because it uses a single molecule of DNA as its building material—can be used to make shapes so small that even traditional light microscopes (the type of microscope you usually see on TV and that you might have used in high school biology class) can't see them. To get a sense of the scale of these objects, consider that Tyrion Lannister/Peter Dinklage is a little over 1 meter tall, while the width of a human hair is approximately 10,000 times smaller (0.0001 meters, or 0.1 millimeters, or 100 micrometers or 4 thousandths of an inch). The smallest origami crane made out of paper measured 1 millimeter wide, while most DNA origami objects are 10,000 times smaller than that (0.1 micrometers or 100 nanometers), roughly the same size as well-known viruses like those that cause AIDS, the flu, rabies and measles (Figure 2). This means that if DNA origami was the width of a human hair, the smallest paper origami crane would be the same size as Peter Dinklage.

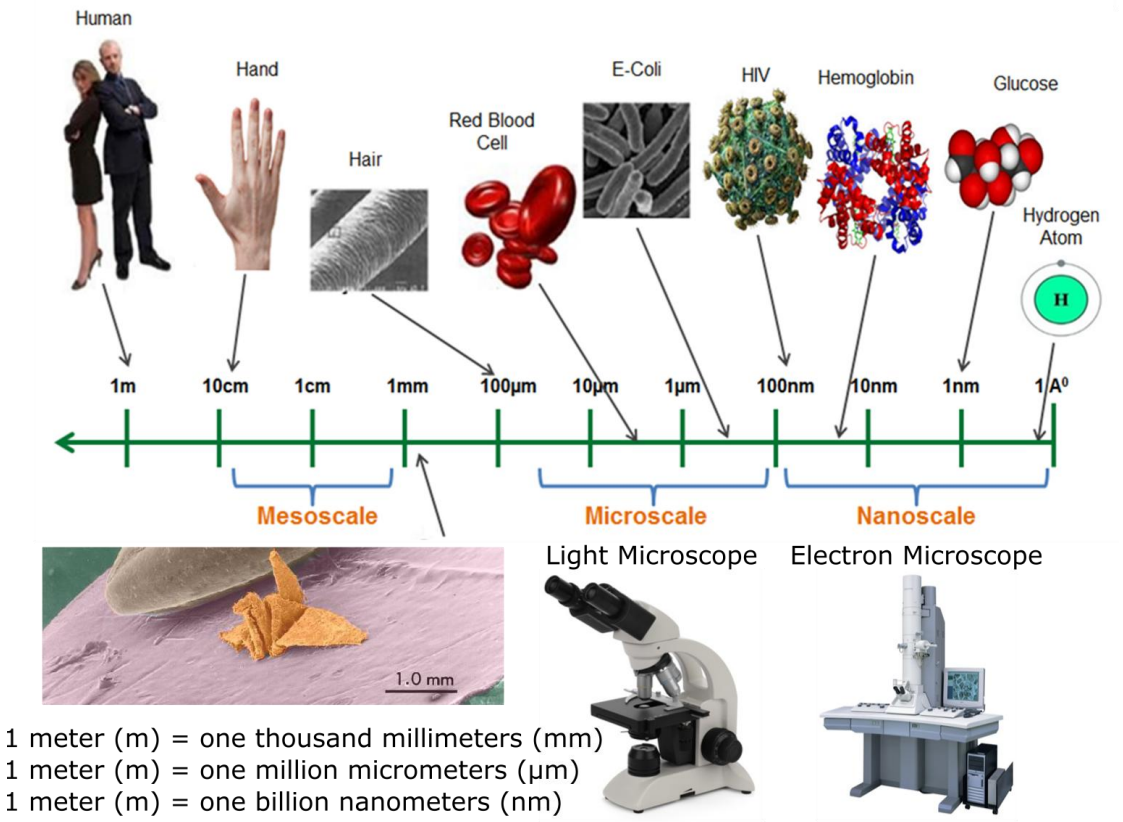

Figure 2. Example objects at different length scales.[5] Microscale objects cannot be seen by eye, but can be seen with a light microscope.[16] Nanoscale objects cannot be seen with a light microscope, but can be seen with an electron microscope.[19] For reference, the cylindrical object behind the origami crane is a grain of rice and the black bar in the bottom right corner of the picture shows how long an object exactly 1 mm in length would be.[21]

Just like paper origami can be decorated with markers, sequins and other add-ons,

DNA origami can be decorated with molecular add-ons such as cancer drugs, gold nanobeads and other useful molecules (Figure 3). Our ability to decorate DNA origami with molecular add-ons has led to breakthroughs in medical imaging technologies, microelectronics, optical material, drug delivery and molecular robotics. While the ability to make such small objects is extremely useful and important, we are limited in

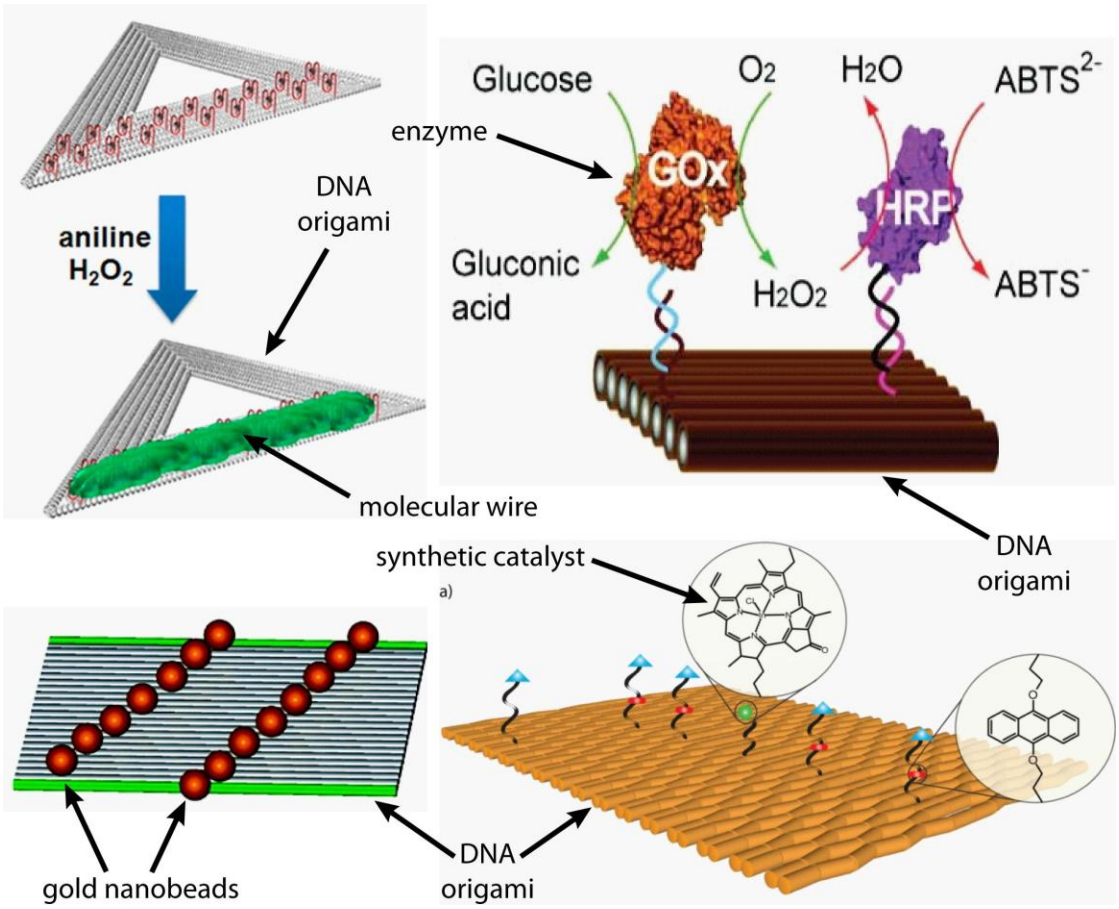

Figure 3. DNA origami objects decorated with conductive molecular wires (top left), naturally occuring enzymes (top right), gold nanobeads (bottom left), and a synthetic catalyst (bottom right). Adapted from references 1-4.

how large the objects made out of DNA are using current techniques. Let's say you want to make an object similar to what can be made with DNA origami, but instead of 100 nanometers long, you need it to be around 10,000 nanometers (or 10 micrometers).

Currently there is not way to do that. DNA origami and other DNA nanotechnology techniques can't make anything that "large". However, the techniques I developed allow us to create similar, but significantly larger objects so that using my techniques you can make your 10 micrometer object out of DNA and decorate it with the necessary molecular add-ons.

5.3 What is DNA Anyway?

To understand how DNA can be used to make these incredible products, we first need to understand its role in nature. As mentioned above, DNA is the molecule that passes genes down from parents to children. Genes are the instructions encoded in the DNA that determines many of your characteristics, such as

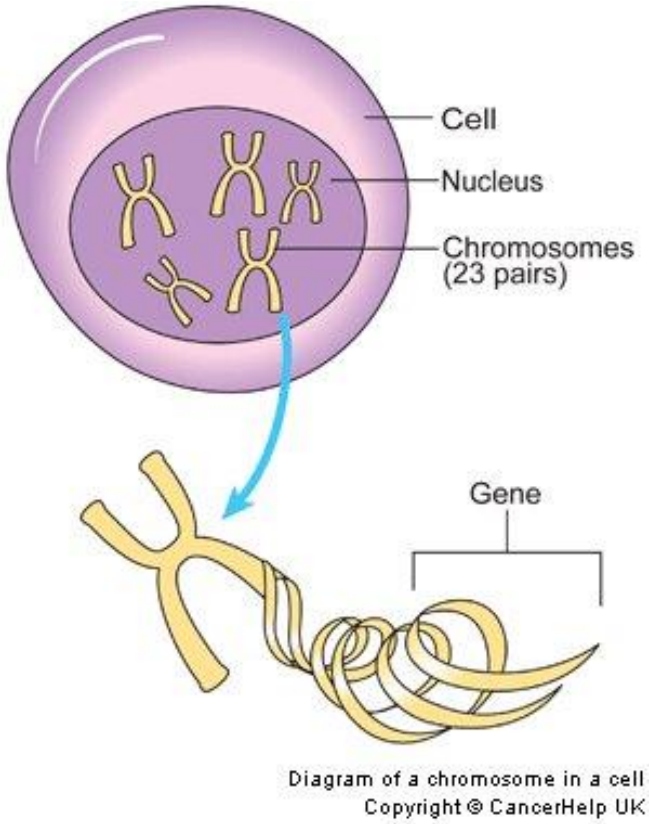

Figure 4. Diagram of a human cell showing the individual DNA molecules (chromosomes) in the nucleus. Reproduced from reference 6.

hair color, eye color, height, etc. To bring you back to high school biology for a second, almost ever cell in your body which contains 46 chromosomes (found in the cell's nucleus, Figure 4). A chromosome is a single molecule of DNA, each of which contains hundreds to thousands of genes. Half of these chromosomes, and thus half of all of your genes, come from one parent, and the other half come from the other parent. Together, all of the *genes* on all of your chromosomes make up your *gene*-ome (better known as a genome).

The reason that DNA can encode information into genes in the first place is that DNA has a "sequence". Maybe you've heard of people getting their genome sequenced by companies such as 23 and Me. Or maybe you remember the "Mr. DNA" scene from the original *Jurasic Park* movie where an animated DNA strand in front of a screen with letters speeding behind him says, "If we looked at screens like these once a second for eight hours a day, it'd take two years to look at the entire strand! It's that long!" Those speeding letters are the sequence of a DNA molecule. If you look carefully at this scene, you will see that there are only 4 letters present: A, C, G and T. This is because the entire human genome (and all other naturally occuring genomes on earth for that matter) is made up of different combinations of these 4 letters. Their arrangement in your genes determines your heritable traits.

5.4 Why is DNA so Special?

DNA can contain so many letters because it is a special type of molecule known as a polymer: a single molecule made up of repeating parts. You can think of a polymer as a long paper chain. Before linking them together, each piece of paper starts as a single,

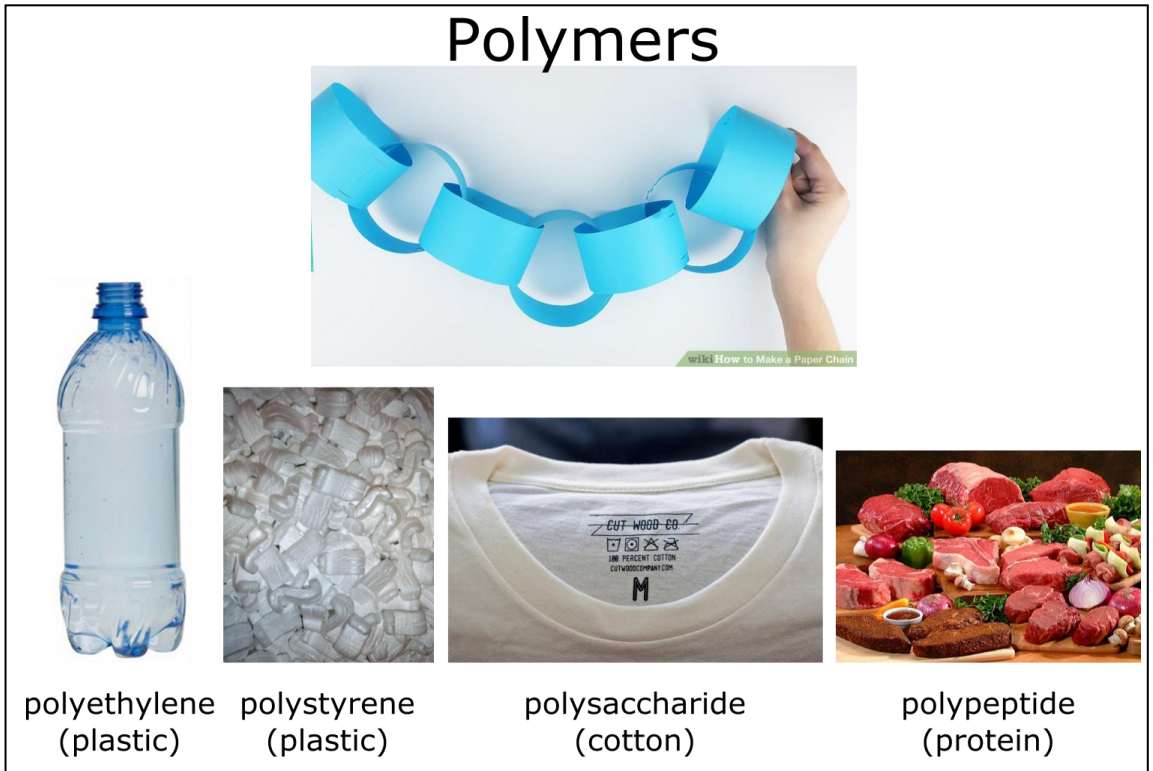

Figure 5. Polymers are individual molecules linked together to form a chain, similar to how individual strips of paper are linked together to form a single chain. Polymers are extremely common in everyday life and make up all plastics such as the polyethylene used to make water bottles, and the polystyrene used to make Styrofoam.[12] Naturally occurring polymers like polysaccharides and polypeptides are also quite common in our lives and make up cotton[18] and protein[20] respectively.

discrete object, but after linking them together, those discrete pieces of paper now make-up a single long chain. Polymers are similarly made by putting together many (poly in fact means "many") small starting molecules (the paper strips) to form a single, long molecule (the paper chain). Polymers are ubiquitous in our every day life (Figure 5). All plastics are made of synthetic polymers. Most water bottles are made of polyethylene, *styr*ofoam is made out of poly*styr*ene. By the way, the "*poly*" in *poly*ethylene, *poly*styrene and many other plastics comes from the fact that these materials are made out of *poly*mers. Naturally occuring polymers are also very common in modern life. Cotton is made out of *poly*saccharides, which are naturally occuring polymers of sugars (although I wouldn't recommend trying to eat your shirt; it turns out that polymers have very different properties, such as taste, from the molecules they started out as). Proteins, including the proteins that are present in food (especially meats and cheeses), are made up of a type of polymer known as a *poly*peptide. Proteins perform many of the functions a cell needs to survive on a daily basis. Those functions include providing much of the structural support of the cell and other parts of your body such as the keratin that makes up your hair and nails. The collagen that binds your organs, such as skin and liver, into distinct objects is also a protein. Notice a common theme among these polymers is that they can all be used for structural purposes to make physical objects.

As another type of polymer, DNA can also be used for structural purposes to make different objects. However, DNA has 2 special properties that make it particularly useful as a building material. First, as you already know, DNA has a sequence. This

means that instead of a single type of starting molecule (the paper strips), the starting molecules that turn into DNA come in 4 “colors”: A, C, G and T, the same letters that were speeding behind Mr. DNA in *Jurassic Park*. In terms of the paper chain analogy, imagine that instead of white strips of paper, you had amber, crimson, green and turquoise strips of paper and you made a 1,000-link paper chain. You could make a nearly infinite number of different chains based on the order you decide for the colored links. Not only that, but if you have a list of the colors of each link (e.g. link 1 = amber, link 2 = crimson, link 3 = green, etc.) you could grab a random link in your paper chain and figure out what number link you are holding by looking at the sequence of colors on the adjacent 10 links and comparing that to the list you have of the colors of each link. We can do this

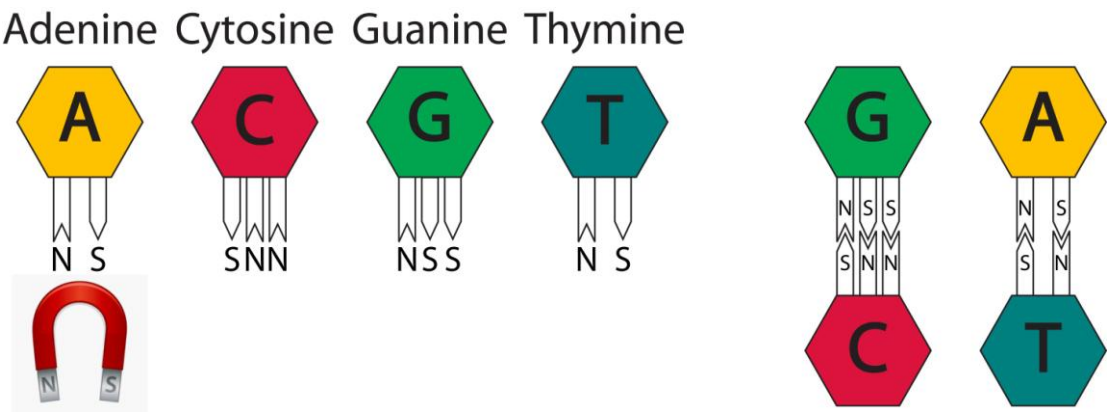

Figure 6. DNA is made up of 4 different “colors” of links: A, C, G and T. Each type of link has a different arangement of molecular magnets attached to them. Because the number and arrangement of poles on each color is different, A can stick only to T, G can stick only to C and vice versa.

same thing with DNA. Because different DNA molecules have different sequences, we can distinguish between them based on their sequences. We can also distinguish different parts of a single DNA molecule because each part also has a different sequence.

The second feature of DNA that makes it so useful as a building material is that it can be double-stranded: a single DNA molecule can actually be made of 2 chains laid parallel to each other (this is why DNA is a *double* helix instead of just a helix). This creates a linear double-stranded molecule (i.e. it forms a single line with no branches). The reason DNA can do this is because each letter that makes up a DNA strand has the molecular version of magnets arranged on it in a unique pattern. And, like normal magnets, these molecular magnets also have poles that attract their complementary pole: north to south for example. Because of this, the A link in DNA only attracts the T link and the C link only attracts the G link (see Figure 6 for details).

This means that if we have 2 DNA molecules that have what's known as complementary sequences (for every A in the 1st molecule, you have a T in the 2nd, for every C in the 1st molecule, you have a G in the 2nd, etc.), and they bump into each other, they will naturally stick together (self-assemble), turning 2 single-stranded DNA molecules into 1 linear double-stranded molecule (Figure 7). However, if the 2 single-stranded DNA molecules are not complementary, they will not stick together when they bump into each other (Figure 7). This is the entire basis for all of DNA nanotechnology and why other polymers can't be used to make the same types of objects. No other known polymer (with the exception of DNA's less stable little brother, RNA) has the ability to

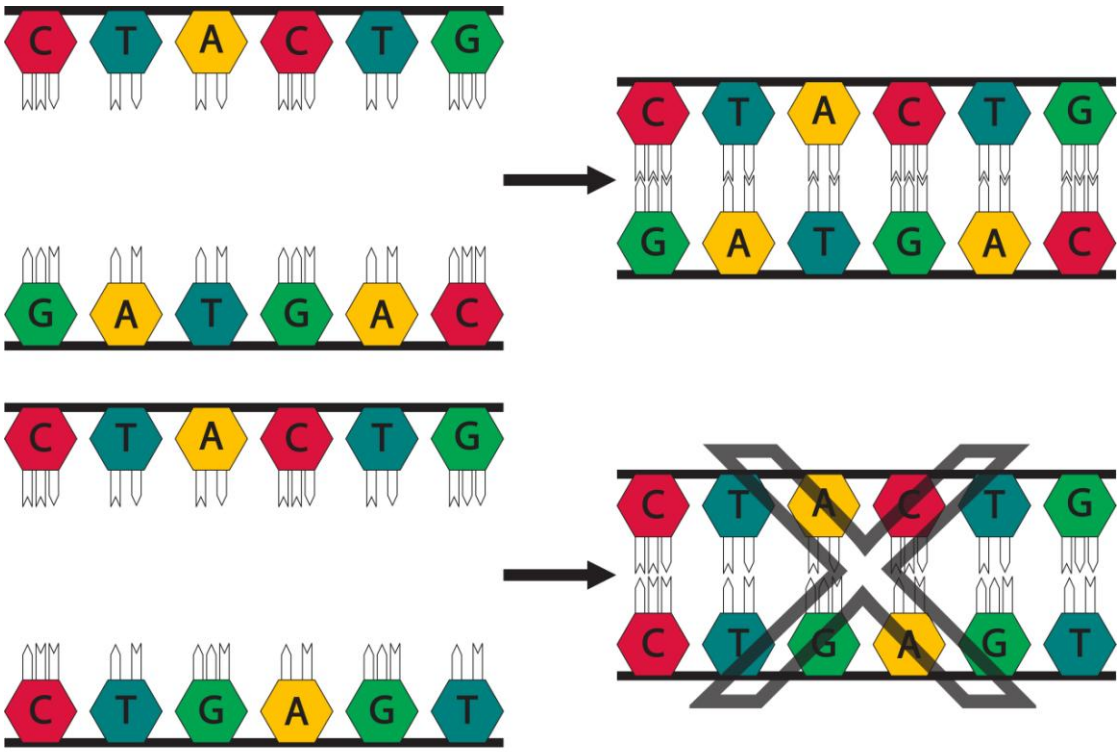

Figure 7. Top: When 2 complementary single-stranded DNA molecules (molecules where all the A's on one strand line up with the T's on the other, the G's line up with C's and vice versa) run into each other, their molecular magnets align perfectly and they stick together to form a normal linear DNA molecule. Bottom: When 2 non-complementary single-stranded DNA molecules bump into each other, because their molecular magnets do not align, they will not stick together.

self-assemble in a sequence-specific manner as it requires a polymer that has a sequence

you can control and that can pair with another molecule. What this means is that you can design DNA molecules that recognize and stick to each other. Imagine you designed 3 DNA molecules to stick to different parts of each other. Part α of molecule 1 (Figure 8) is

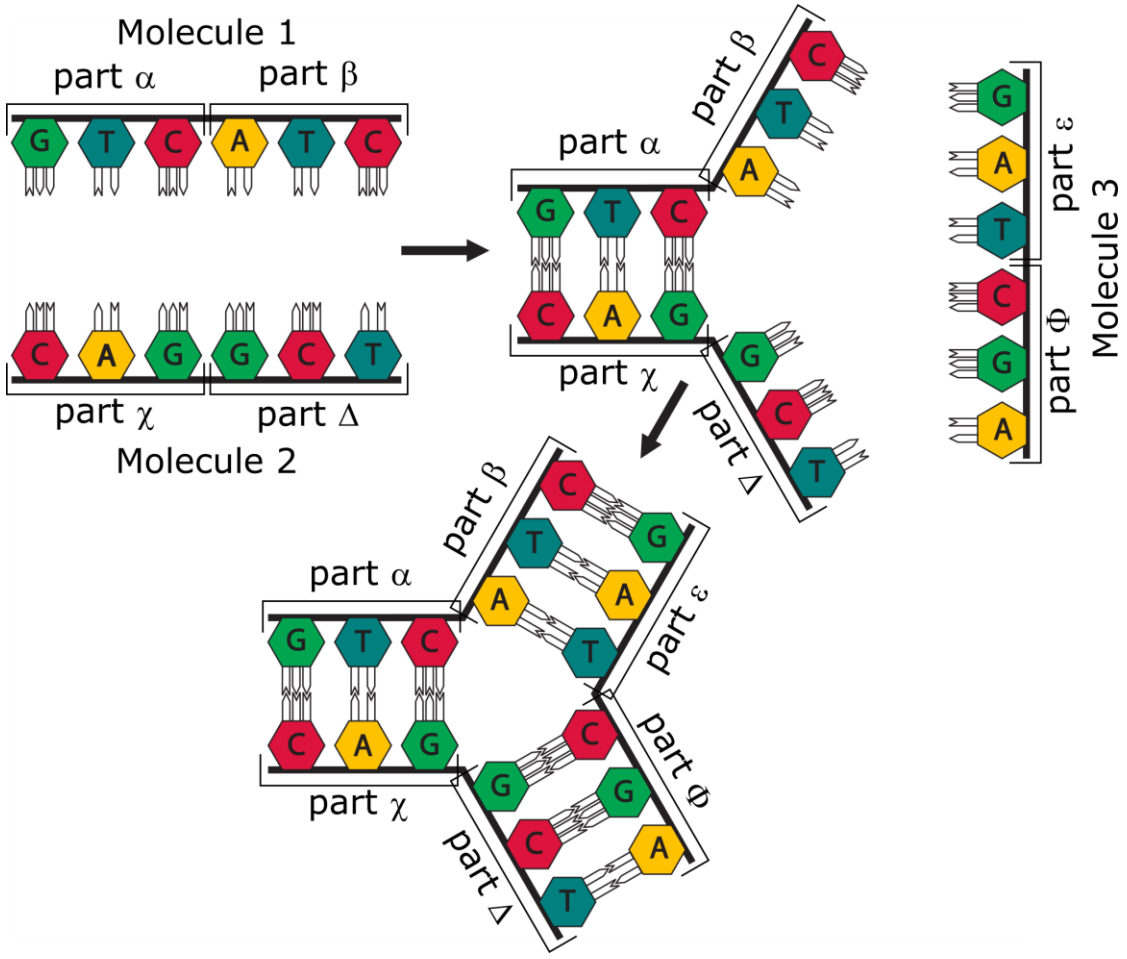

Figure 8. If 2 single-stranded DNA molecules are partially complementary, then the parts that are complementary will stick together, but the parts that aren't complementary won't, leaving those parts single-stranded, thus allowing another single-stranded DNA molecule that is complementary to those regions to stick and form a Y-shaped junction.

designed to stick to part χ of molecule 2, but part β of molecule 1 is designed to stick to part ε of molecule 3 and part Δ of molecule 2 is designed to stick to part Φ of molecule 3. This means that by combining these type of DNA molecules, we can start to make what are known as DNA junctions: Y-shaped (with 3 “arms”) or X-shaped (with 4 “arms”) DNA molecules made up of 3 or 4 DNA strands each around 50 "links" long rather than the 2 strands that make up normal linear DNA (Figure 8).

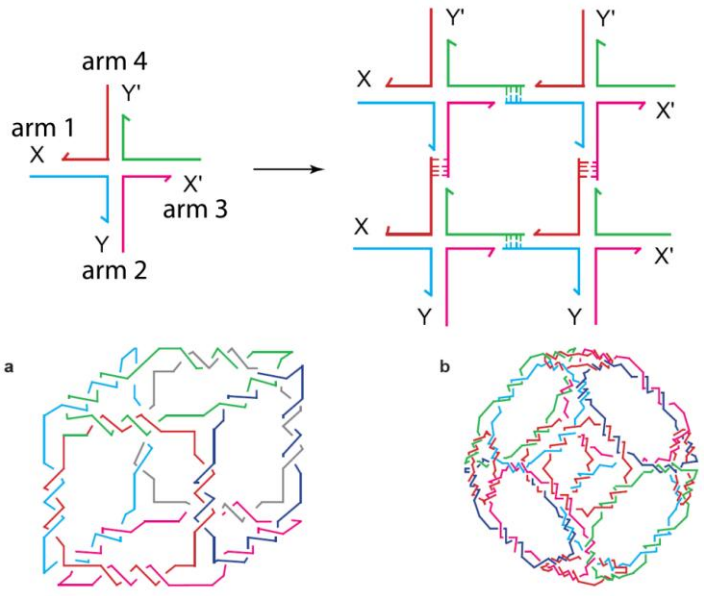

Figure 9. Top: how DNA junctions can come together to form objects. Bottom: example 3-dimensional objects (cube left, icosohedron right) made by sticking different junctions together. Each vertex on each object is made of a different DNA junction. Adapted from reference 14.

DNA nanotechnology got its start in the 1980's using DNA junctions that included single-stranded "sticky ends" at the end of each arm of the junction. These are called sticky ends because of single-stranded DNA's ability to recognize and stick to other single-stranded DNA molecules of a specific sequence as described above. Because DNA junctions have these

single-stranded sticky ends, multiple junctions could be combined to self-assemble objects, including simple polyhedra such as a cube (6 sides) or icosahedron (20 sides, Figure 9). This is similar to the way multiple single-stranded DNA molecules were combined to form the DNA junctions in the first place. Unfortunately, objects made using DNA junctions are limited to ~10 nm across (really, really, really, really small, Figure 2). The one exception to this is if the DNA junctions are designed so that arm 1 on the junction is attracted to its own arm 3 and arm 2 is attracted to its own arm 4. When this happens, each junction will stick to 4 other junctions that are identical to itself. Then those 4 junctions will hybridize to 3 other junctions identical to themselves, and this process will repeat itself over and over again. This forms a repeated structure where no matter which part you zoom in on, the molecular structure looks the same. Everyone is actually very familiar with these types of structures, which are better known as crystals (Figure 10). DNA crystals can form structures out of DNA so large that you can see them without a microscope. However, it is impossible to distinguish between different parts of the crystal because all parts are made of the same DNA junction repeated over and over. This inability to make structures >10 nm across—without making a repetitive

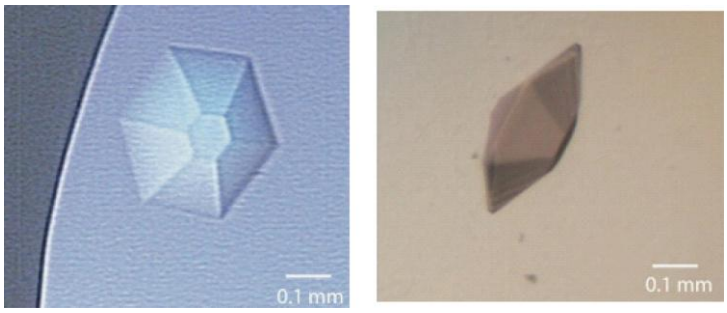

Figure 10. Example crystals made out of repetitive DNA junctions. Adapted from reference 13.

structure—made DNA nanotechnology a rather narrow field of study, even among scientists. This changed with the invention of DNA origami in the mid-2000's.

Now that we have some background on the structure of DNA and DNA self-assembly, I can explain how DNA origami can make the wide variety of relatively large shapes seen in Figure 3 (~10 times larger than the shapes made with DNA junctions). Instead of using just the short DNA molecules with ~50 links used to make the DNA junctions, DNA origami includes a relatively long single-stranded DNA molecule with ~7,000 links (known as the scaffold strand) as its starting material. Remember that it must be single-stranded because that way the molecular magnets on each link are exposed and can stick to another single-stranded DNA molecule of the correct sequence. We create DNA origami by folding this ~7,000 link long scaffold strand using lots of short single-stranded staple strands (DNA molecules ~30 links long). These staple strands are designed so that half of the staple is attracted to 1 part of the scaffold strand, but the other half of that staple strand is attracted to a completely different part of the scaffold strand. When the staple strand runs into the scaffold strand, that 1 staple strand will stick to 2 areas on the scaffold strand, thus stapling those 2 areas together and folding the scaffold strand over on itself. Imagine that you had to make a paper origami object without being able to crease the paper, but you could use a stapler to permanently keep any 2 parts of your paper together. By adding hundreds of ~30 link staple strands to a single ~7,000 link scaffold strand, you can fold the scaffold strand into any shape you want, just by changing what 2 parts of the scaffold strand the staple strands you add are attracted to (Figure 11).

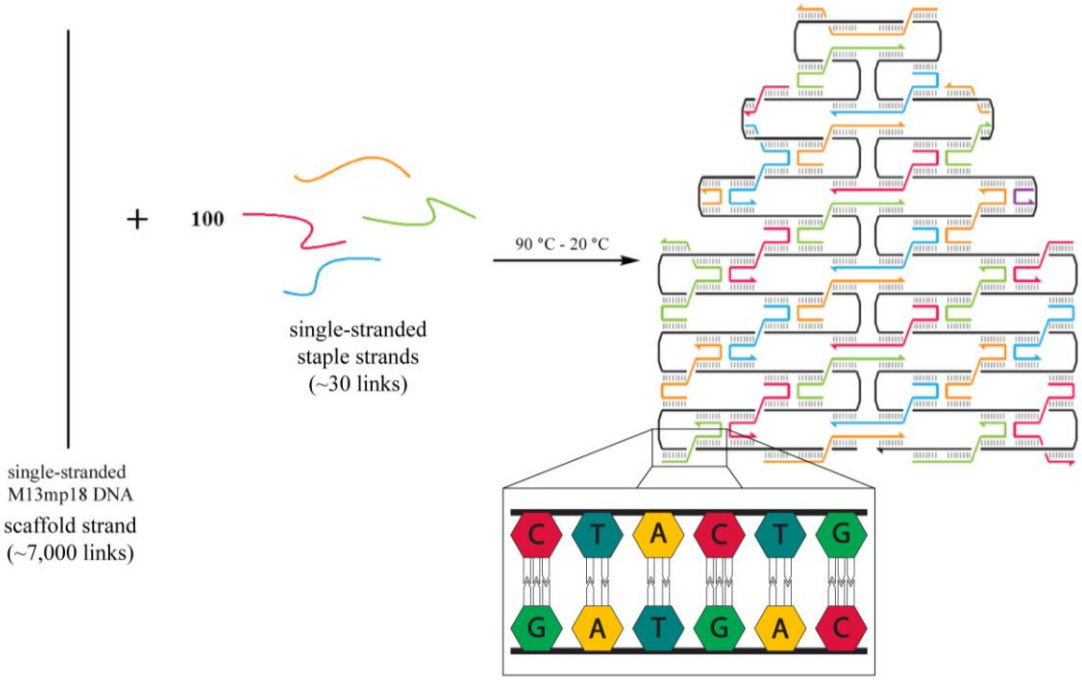

Figure 11. DNA origami works by taking a long single-stranded scaffold strand (~7,000 links long) and adding short, single-stranded staple strands (~30 links long) where each half of each staple strand sticks to a different part of the scaffold strand, thus folding the scaffold strand into a specific shape. Adapted from reference 7.

And because the scaffold strand is a single, continuous strand of DNA, each part of the object has its own sequence and therefore its own address. This is what allows DNA origami to be decorated at precise locations with a variety of different add-ons, since if you add a single-stranded DNA molecule to your add-on, it will stick only to the part of the DNA origami object that has the correct sequence.

The ability to make objects of any shape and decorate them at precise locations with a variety of different molecular add-ons has revolutionized the field of DNA

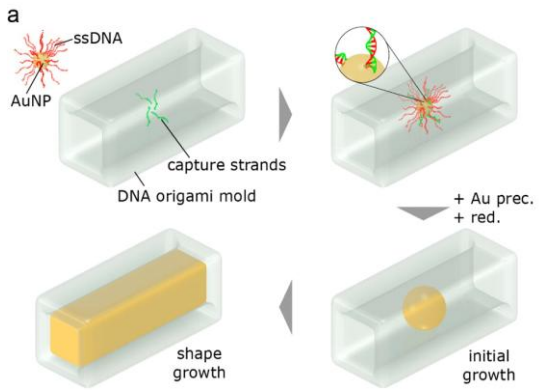

Figure 13. A hollow rectangular DNA origami tube with single-stranded DNA (ssDNA) molecules on the inside captures a gold nanobead (AuNP, upper left) with complementary single-stranded DNA molecules attached to it. By growing the size of the gold nanoparticle, it takes the shape of the inside of the DNA origami, thus allowing the creation of gold nanoparticles of a specific size and shape. Adapted from reference 17.

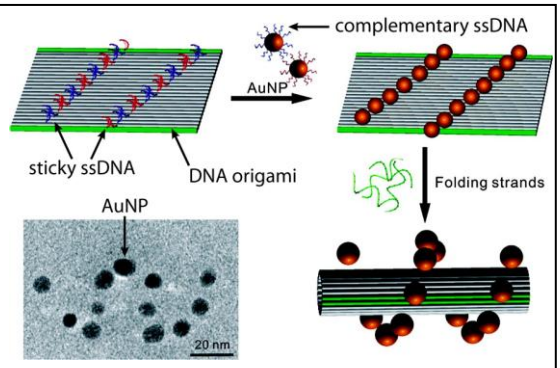

Figure 12. A DNA origami rectangle with 2 tracks of sticky single-stranded DNA (ssDNA) molecules capture gold nanoparticles (AuNP) with complementary ssDNA to create tracks of gold nanoparticles. The DNA origami can then be rolled up, creating a spiral of gold nanoparticles. Bottom left: DNA origami/gold nanoparticle spiral image taken using an electron microscope. Adapted from reference 4.

nanotechnology. DNA origami has been used as a mold to create other objects such as gold nanoparticles of any shape desired (Figure 13). This could have applications in such fields as drug synthesis and medical imaging technologies. It has also been used to template gold nanoparticles into specific shapes (Figure 12) that have been shown to bend light in interesting ways, potentially allowing for it to be used for optical materials similar

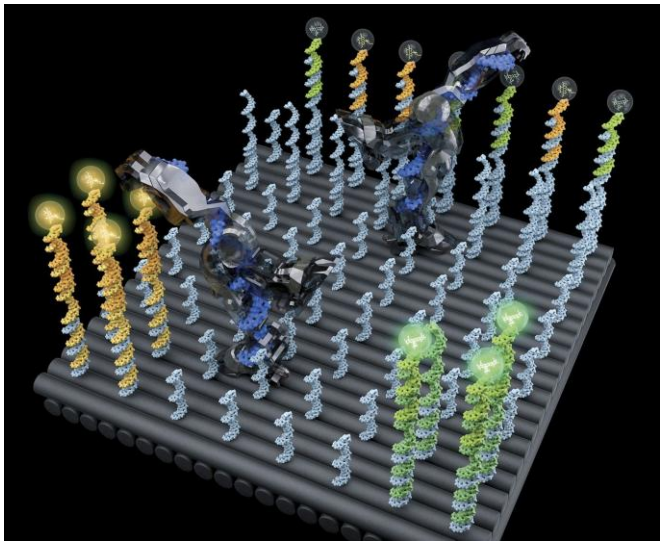

Figure 14. Artist's depiction of a pair of molecular cargo sorting robots made using DNA origami. Adapted from reference 15.

to the liquid crystals used in many computer screens. DNA origami has even been used to create molecular robots that can sort single-molecules (Figure 14).

While DNA origami can be used for a variety of different purposes, its greatest strength is also its greatest weakness. DNA origami works because it has a single-stranded DNA molecule as a scaffold. Remember that each link in the DNA chain has molecular magnets that can pair with the molecular magnets of another DNA chain if that chain is the right sequence. The size and complexity of the objects made using DNA origami depends on how many links the scaffold has. The more links, the larger the DNA origami. Unfortunately, single-stranded DNA is fragile, and the longer it gets, the more difficult it is to keep intact. This is because if even 1 link breaks, you no longer have just 1 single-stranded DNA molecule —you have 2, shorter, single-stranded DNA molecules (Figure 15). The more links there are, the more likely it is that 1 of them will randomly break. This limits the size of the DNA scaffold used in DNA origami, which in turn limits the size of the DNA origami object made with it, and that limits the applications DNA origami can be used for.

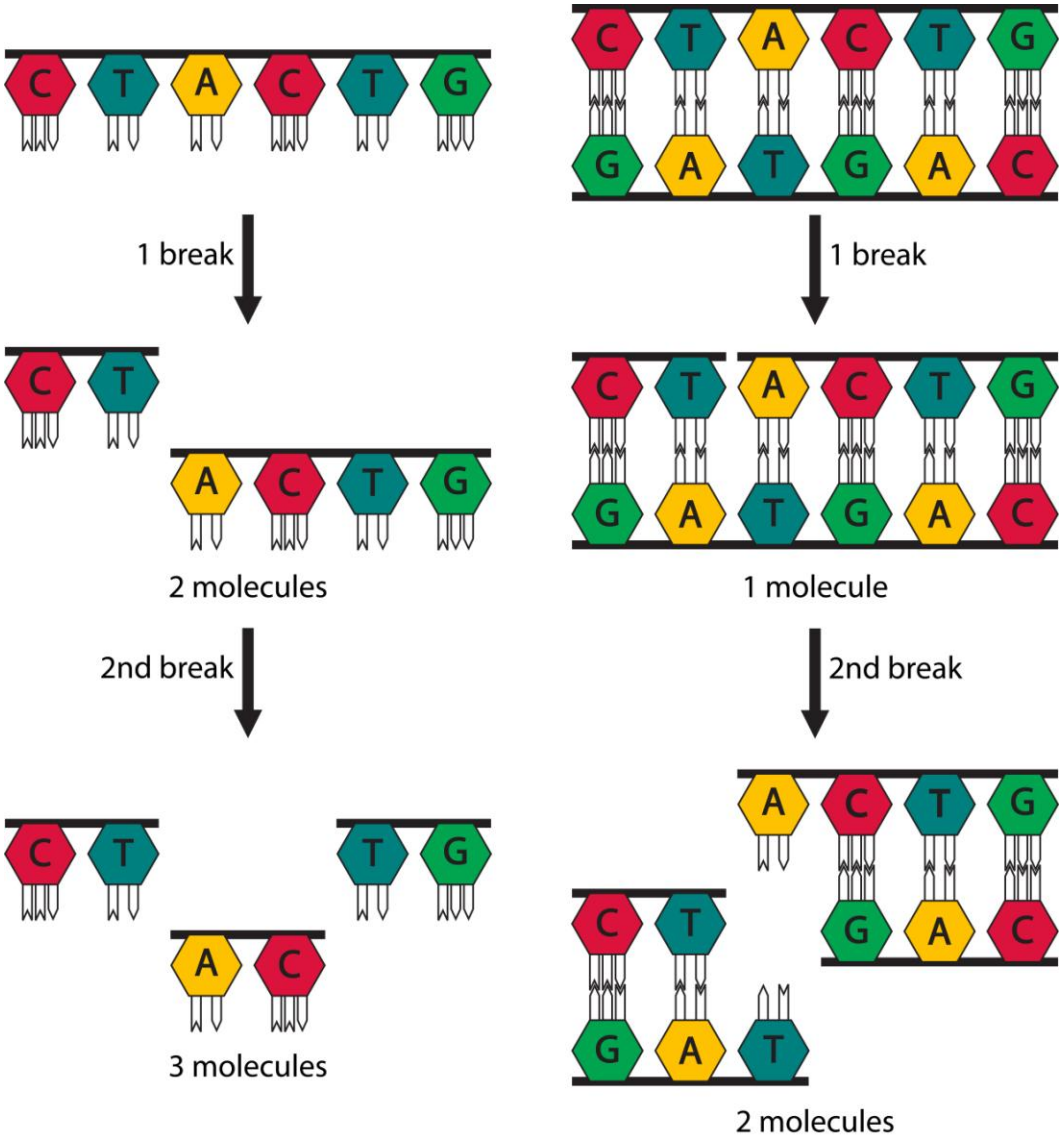

Figure 15. Left: If you break a single-stranded DNA molecule once, the molecule breaks into 2 pieces. If you break it again, you get 3 molecules. Right: If you break a double-stranded DNA molecule once, the molecule stays together and you maintain a single molecule. It is only if the molecule is broken a 2nd time on the opposite strand and close to the site of the first break, that the molecule breaks apart.

5.5 Making Objects out of Double-Stranded DNA

Because double-stranded DNA is, well, double-stranded, it is much more resistant to breaking than single-stranded DNA. This is because in addition to the links that keep each strand together, double-stranded DNA is also kept together by the molecular magnets on each link. While each magnet is too weak to keep the DNA by itself, if you have >50 molecular magnets right next to each other, together they make a strong bond between the 2 strands of DNA. This means that even if 1 of the links on 1 of the DNA strands breaks, the double-stranded DNA molecule will still stay together (Figure 15). Even if several links on both DNA strands break, as long as there are >50 molecular magnets between the breaks, the DNA molecule does not break apart. This makes double-stranded DNA much more stable than single-stranded DNA. This added stability can also be seen in nature, where the longest known naturally occurring single-stranded DNA molecule is ~25,000 links long, which if you stretched it out would be ~8.5 micrometers (8500 nanometers) long (see Figure 2 on page 468 for a refresher on how big this is). Compare that to the largest double-stranded DNA molecule in the human body. This molecule is ~224 million links long and would stretch out to ~76 mm (~3 inches). If double-stranded DNA could be used as the scaffold instead of single-stranded DNA, that would allow us to create much larger and more complicated objects. Unfortunately, if DNA is double-stranded, that means that all of its molecular magnets are already paired with another DNA strand. Even if you add a third DNA strand, it can’t access the

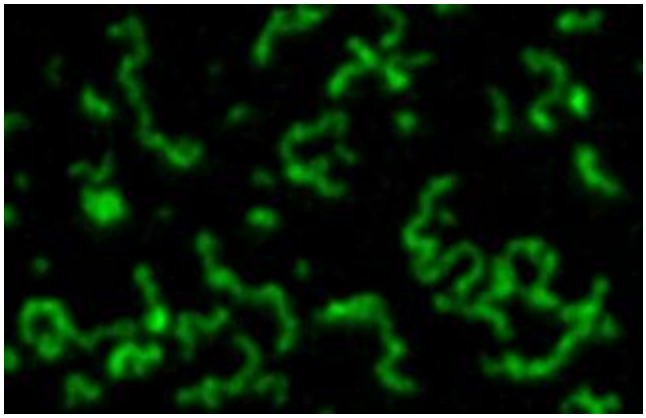

Figure 16. Each green squiggle is a single-molecule of double-stranded DNA stained with a fluorescent dye and imaged under a microscope. Each DNA molecule would be ~16 micrometers long if it was stretched out.

molecular magnets on the double-stranded DNA that would allow it to stick to the specific sequence it's looking for.

Before I get into how I was able to get around this problem, I would like to briefly describe what I see when I look at double-stranded DNA. Because double-stranded DNA is so long, I can use a standard microscope similar to the ones you see on TV to see individual DNA molecules. However, while double-stranded DNA is very long, it is also very thin: ~2 nm across. This is so thin that it is impossible to see in its normal state. Fortunately, there are fluorescent dyes (similar to the dyes used in glow sticks) that will stick to double-stranded DNA, and when I look at this dyed DNA under the microscope, I see a bunch of bright green squiggles that look like glowing green strings that have been thrown on the floor (Figure 16). This means that if I fold the DNA into a new shape (imagine tying those green strings into a bow before throwing them on the floor), I can actually look at the DNA under the microscope and see if the DNA is folded into the shape I want or not.

Of course, in order to look at folded DNA under the microscope, I first had to figure out ways to modify the double-stranded DNA to fold it like we can fold single-stranded DNA. I first made a simple model system I could fold by hijacking nature's method of DNA modification using an enzyme known as a *polymer*ase (because it forms the *polyme*r DNA) that nature has designed to add new links to DNA. This allowed me to add links to my DNA chain that were modified with the molecular version of Velcro loops at a specific sequence of DNA. On the particular DNA molecule I was working

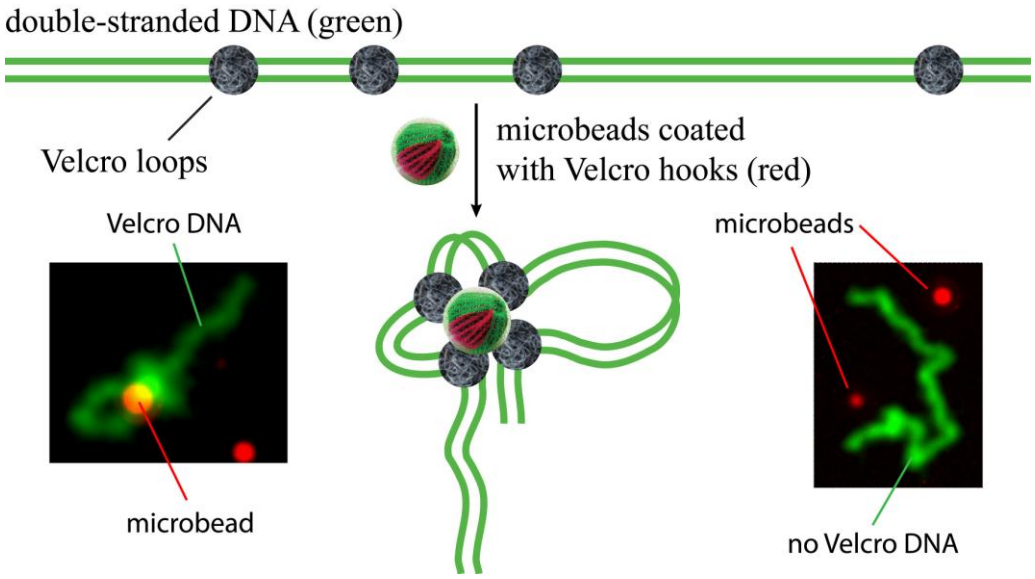

Figure 17. By adding molecular Velcro to DNA, a microscopic bead (microbead) coated with velcro loops can fold the DNA by sticking to the sites that have Velcro while ignoring the parts that don't. This folds the DNA into a rosette that can be seen under the microscope. On the left is an image of a DNA molecule (green) with Velcro folded around a bead (red). On the right is an image of a DNA molecule that doesn't have Velcro and thus does not stick to the beads that can also be seen in the image.

with, this put 4 Velcro patches at specific places on the DNA. By mixing in a red fluorescent microscopic bead (microbead) coated with the molecular version of Velcro hooks—which will bind to the parts of the DNA with the Velcro loops but not to any other part of the DNA—the DNA with the Velcro loops should fold into a specific shape, in this case a rosette (Figure 17). Imagine my delight when I looked at this DNA under the microscope and saw something that no one else in the world had ever seen before: a folded, double-stranded DNA rosette, very different from the linear squiggles I saw when I added the Velcro microbead to DNA without the molecular Velcro loops (Figure 17). While this made my day, this method of folding is nowhere near as controllable as DNA origami because anywhere that had the Velcro loops would bind to my bead. I wanted to be able to choose exactly which parts of the DNA would be bound together so that I could create a molecular erector set out of DNA.

To do this, I needed to find a way to add single-stranded DNA to a double-stranded DNA molecule so that I would have the physical stability of double-stranded DNA *and* the molecular recognition through sequence-specific attraction of single-stranded DNA. The way I did this was to build my own DNA molecule so that I could add Y-junctions similar to those described above (page 478) to specific parts of my DNA molecule. This allowed me to place single-stranded DNA "flaps" of unique sequences on different parts of my double-stranded DNA molecule ~48,500 links (~16 micrometers) away from each other with ~20,000 links on either side of each flap (Figure 18). By having 2 flaps of different sequences on different parts of the DNA molecule, I could add

a single-stranded DNA “strap” that attracts both flaps, and thus fold the DNA to create an object reminiscent of the bolo ties of traditional western United States fashion. Of course, if it were that simple, it wouldn’t be worth getting a PhD for and it wouldn’t have taken me 6 years to get it to work.

Just like normal magnets, molecular magnets attract each other only when they are quite close to each other. This means that for the strap to find the flap in a reasonable

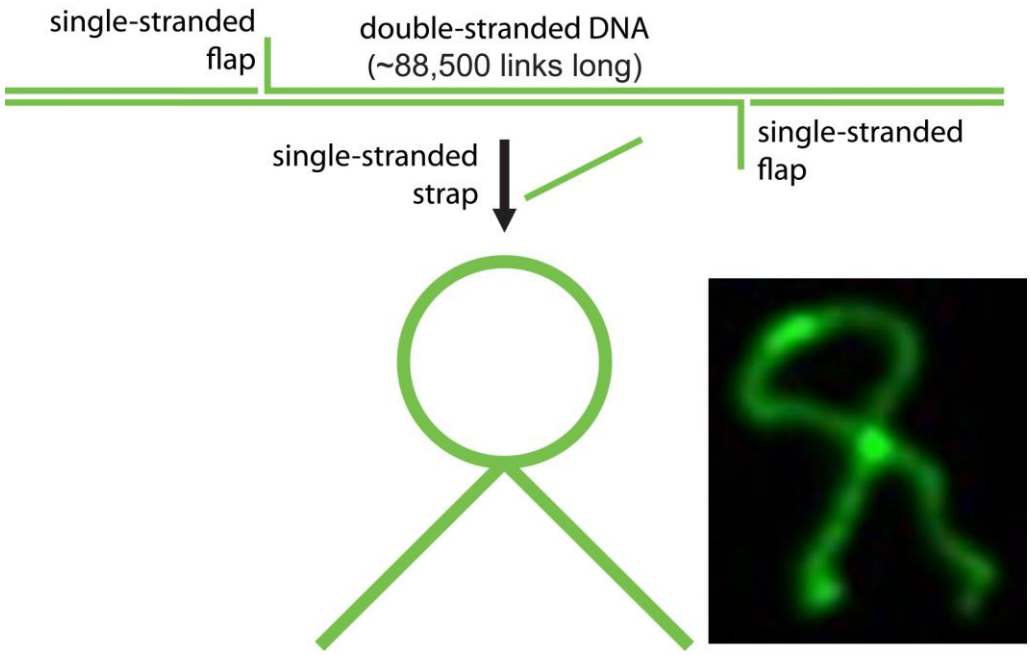

Figure 18. By building an ~88,500 link double-stranded DNA molecule with single-stranded flaps of unique sequences ~20,000 links away from the ends of the molecule, we can add a single-stranded strap that’s complementary to both flaps and fold the double-stranded DNA into a bolo-tie shape similar to how DNA origami folds single-stranded DNA into different shapes. However, our bolos are ~5 micrometers across, ~500 times larger than the objects typically made with DNA origami.

amount of time, I need to add hundreds of straps for every flapped double-stranded DNA molecule I have. Imagine you were playing hide and seek in a giant sports stadium. With only 1 person seeking, you'd be hidden a long time, even if both of you were wearing complementary magnets. But if hundreds of people were looking for you, it wouldn't take nearly as long. However, this actually presents a problem, because I need the same strap/seeker to find both flaps/hiders. If 2 different straps/seekers find the 2 flaps/hiders, then the 2 straps would stick to the 2 flaps. That would prevent any strapping because the straps are not complementary to each other and thus would not stick to each other even if they do run into each other. And even though the 2 flaps are tethered together by the double-stranded DNA molecule, this molecule is so big, that it is like the 2 flaps are in the same section of the stands, not necessarily in the same row.

To solve this problem, I had to turn to the only other molecule that is has a controllable sequence that can pair with another molecule of a specific sequence: DNA less stable little brother, RNA. You can think of RNA as exactly the same as DNA, except that instead of its paper chain links being held together by tape, they're being held together using a really old, brittle glue. I'd warn you not to even sneeze because that would cause it to break apart. By adding an RNA "blocker" that has the same sequence as 1 of my flaps to the strap, I was able to block the strap from sticking to that flap while still allowing it to stick to the other flap (Figure 19 step 1). Imagine that in our game of hide and seek—now with 2 hiders—that I put special glasses on the hundreds of seekers so that they could see only 1 of the concealed players rather than both.

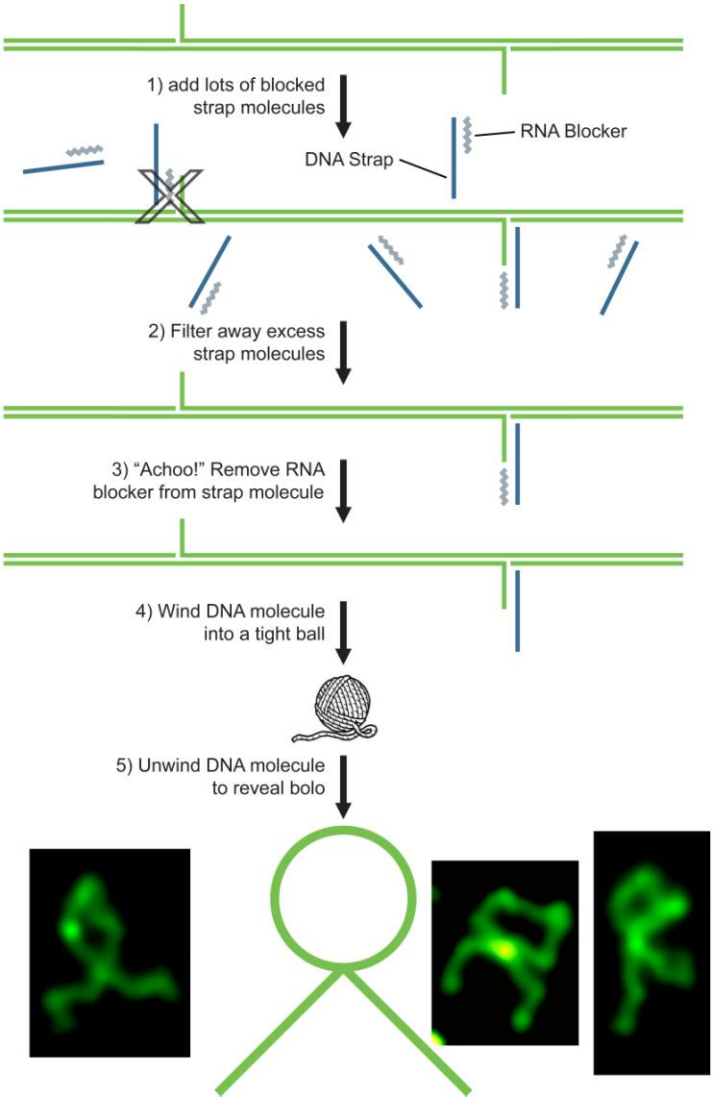

Figure 19. Double-stranded DNA can be folded if it has single-stranded flaps by adding lots of complementary single-stranded straps that have one half blocked with RNA so that it can only stick to 1 flap. Filtering away the extra strap molecules, removing the RNA blocker and then winding the DNA into a tight ball allows the blocked part of the strap to find and stick to the 2nd flap so that when you unwind the DNA, it is folded into the desired object.

Because the staps are so much smaller than the flapped double-stranded DNA (the strap is ~15 nm long while the flapped double-stranded DNA is ~30 micrometers long, 2,000 times larger), I could filter the extra straps away from the flapped double-stranded DNA molecules so that the only straps left are those stuck to the 1 flap of the double-stranded DNA (Figure 19 step 2). At this point, I could figuratively sneeze at my RNA blocker to make it fall apart (Figure 19 step 3). This takes away the special glasses on the seeker who is left in the stadium (and who is permanently stuck to one of the hiders) so that he/she can see the other hider. However, because the DNA connecting the flaps together is so big (imagine that the 2 hiders are connected by a mile-long rope), even with the seeker being able to see the 2nd hider, it would still take a long time for that 1 seeker to find the 2nd hider.

To speed this process up, I had to add the equivalent of a yarn ball winder for DNA. This would transform the DNA from a loose ball into a tight ball. This means that our strap/seeker, instead of searching the entire stadium for the 2nd flap/hider, can use the rope tethering the hiders together to pull the 2nd hider to itself, drastically increasing the chance that the seeker will find that 2nd hider (Figure 19 step 4). The best day of my graduate experience was when I unwound this DNA, looked at it under the microscope and saw a bunch of fluorescent green bolos staring back at me (Figure 19 step 5). I was so excited I had to call my parents and let them know the good news. After 6 years of trying to be able to fold double-stranded DNA into microscale objects, I had finally succeeded.

Using the methods I have developed, it should now be possible to take a double-stranded DNA molecule that has lots of flaps on it and fold it into really complicated objects similar to those made with DNA origami, but hundreds of times larger. And not every flap needs to be strapped some of them can be used to decorate these objects the same way we decorate DNA origami—with molecular wires to create shaped wires potentially useful for microelectronics, with gold nanoparticles to create novel optical materials, or, because they are so large, with DNA origami to create DNA superstructures that have different sites for different purposes (Figure 20). The possibilities are endless.

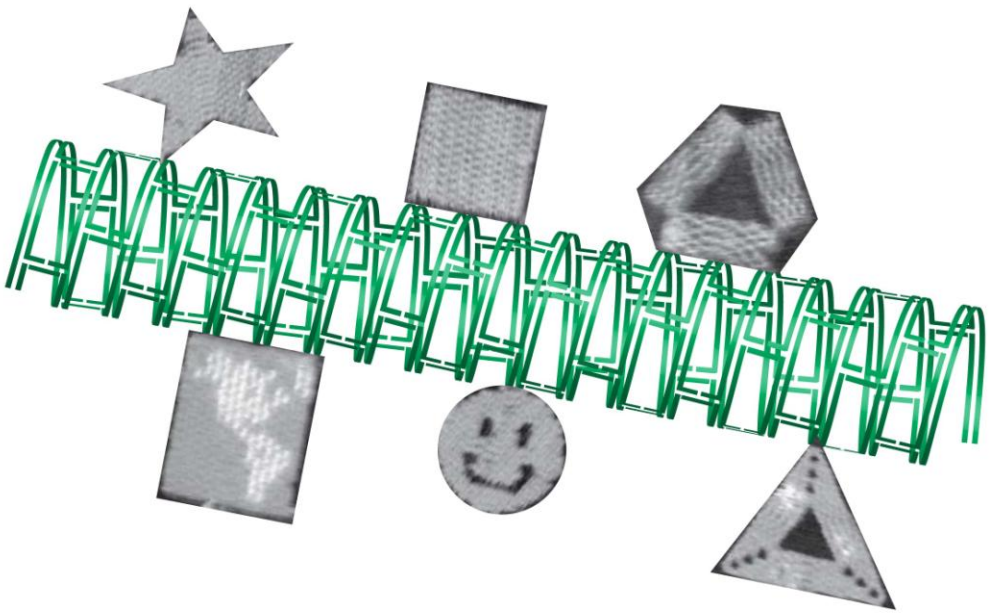

Figure 20. Using flaps and straps, it should be possible to fold large, double-stranded DNA molecules into complex shapes, such as a spiral, that can be decorated with different objects, even objects made from DNA origami. DNA origami images adapted from reference 7.

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Acknowledgements

I would like to thank my parents, professor Shakhashiri and the science communications program of the Wisconsin Initiative for Science Literacy for their guidance in making this chapter as clear as possible for non-scientists. I appreciate the time and effort they put into helping me. This chapter is more accessible to everyone because of it.