IJHSR
ijhighschoolresearch.org
RESEARCH ARTICLE

DNA Origami: Its Evolution and Application in Nanotechnology

Kavi A. Ullal

UWC-USA High School, State Rte. 65, Montezuma, New Mexico, 87731, USA; kavi.ullal@gmail.com

ABSTRACT: DNA origami is the construction of DNA based nanostructures, either through folding a single-stranded DNA scaffold or by creating tile-based structures. This paper reviews the conceptual advances and seminal research in this emerging field, and its application in areas like medicine and biotechnology. DNA origami has its roots in the discovery of the Holliday junction, a temporary branched nanostructure formed during meiosis. Nadrian Seeman pioneered DNA nanotechnology by using immobile Holliday junctions along with sticky ends and double-crossover motifs to create elementary structures. In the early 2000s, scientists transitioned towards using single-stranded DNA as scaffolds folded by several shorter oligonucleotides. Subsequently, researchers used shorter single-strands to create tile-based structures, equivalent to pixels in a molecular canvas. Binding between tiles on the canvas was selectively prevented through the use of edge protectors to arrive at a desired shape. More recent techniques such as fractal assembly have combined elements from the single strand scaffold and tile-based methods. DNA origami design has been further revolutionized by the development of software packages such as CaDNAno and Adenita. These packages will accelerate the application of DNA origami in nanofabrication and drug delivery, making it one of the most exciting and innovative areas of nanotechnology.

KEYWORDS: Biochemistry; Structural Biochemistry; Nanotechnology; DNA Origami; Holliday Junctions; DNA Scaffold.

Introduction

Scientists have long dreamt of being able to control the composition and structure of materials on the nanoscale. In the past two decades, DNA has emerged as an important building block for creating nanostructures. Scientists first considered DNA for this purpose due to its 2 nm diameter, structural repetition arising from its 3.37 nm helical pitch, and mechanical stiffness (with a persistence length of around 50 nm).[1] These physical characteristics in combination with the programmable nature of its molecular makeup make it appealing for nanoscale construction. Many DNA based structures already occur naturally during cell division and other biological processes and can serve as a mechanistic basis for the artificial synthesis of nanomaterials.

DNA origami is the construction of DNA based 2D and 3D nanostructures, typically by folding a long single stranded DNA (scaffold) or using it to create tile-based structures. This design process has been transformed by the advent of new software packages such as CaDNAno and Adenita.[2,3] These software tools are likely to make 3D DNA nanofabrication accessible to scientists in a wide variety of fields.

Recent innovations in DNA nanotechnology have exciting potential application in fields such as microbiology, photonics, and medicine.[4-6] Although research regarding the use of DNA origami commercially is in the early stages, it has shown itself to be promising in the lab setting. DNA origami has been used to deliver drugs to cancer tumors in mice with high specificity.[4] Scientists have also experimented with the construction of a controllable, 3D DNA origami box for application in drug-delivery systems.[7] DNA is also being proposed as a medium for nanocomputing, and early experiments have shown that it can do so.[8] DNA origami appears likely to be a growing field for innovative research and has developed significantly since the original breakthroughs in DNA nanofabrication in the 1980s.

This paper is based on a literature review by the author of key publications in the field of DNA origami and nanofabrication. It summarizes the key intellectual advances and applications in the field. Existing literature reviews on DNA origami either focus on specific sub-topics or are written for specialized advanced researchers. This paper is written for high school or college students with an advanced interest in structural biochemistry, and for scientists who work in other fields who want an introductory primer on DNA origami. Making the advances in DNA origami more accessible to a wider audience will broaden the interest and possibilities in this fast-growing domain.

Methods

The Structure of DNA:

There are two approaches to forming nanoscale structures: top-down and bottom-up construction. Top-down construction is when the amount of material used for patterning and feature size is reduced to the required dimensions to create the desired shape. This differs from bottom-up construction, where structures are created by controlling the assembling atoms in the desired locations of interest. DNA has emerged as an important component of bottom-up nanofabrication techniques.

The unique nature of DNA makes it highly suitable for use in the construction of nanoscale structures. DNA consists of two strands intertwined and running in anti-parallel orientations. The strands are connected through pairs of nitrogenous bases. The four bases are Adenine, Thymine, Guanine and Cytosine. Adenine and Thymine always bond with each other through two hydrogen bonds, while Guanine and Cytosine pair through three bonds, resulting in a higher melting point. The

DOI: 10.36838/v4i3.18

process of forming the double stranded molecule through base pairing is referred to as hybridization. Another feature of DNA is the ability to connect two DNA molecules by using strands that are of slightly different lengths, a process known as sticky end cohesion (Figure 1). If the 'overhangs' from two different helices are complementary, they can be induced to bind, thereby enabling the creation of larger lattice structures. The highly programmable nature of DNA means that it can be used for creating origami nanostructures.

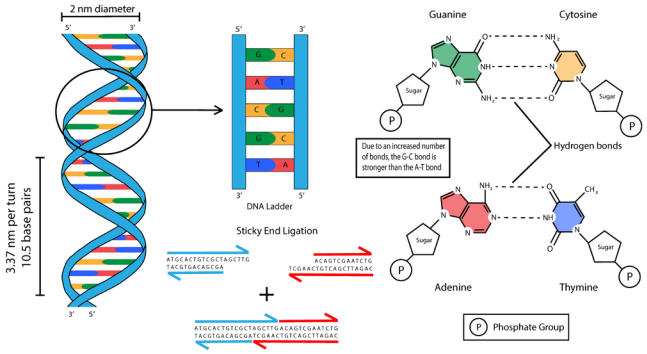

Figure 1: DNA consists of two strands running in anti-parallel orientations. These strands are connected through four different types of nitrogenous bases: Adenine, Thymine, Guanine, and Cytosine. These properties make DNA highly programmable.

Holliday Junctions as Nanotechnology Building Blocks:

The origins of bottom-up DNA origami can be traced back to the discovery of the Holliday junction. A Holliday junction is a naturally occurring temporary DNA nanostructure that forms when aligned strands of DNA break and cross over one another, prior to cell division.[9] Holliday observed that four single DNA strands are centered around a branch point to form a junction-like shape with four arms. (Figure 2)

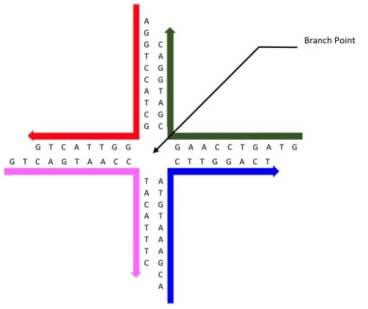

Figure 2: Illustration of a Holliday junction. There are four DNA single strands located around a central branch point.

In the early 1980s, Nadrian Seeman, regarded as a pioneer in the field of DNA nanotechnology, began using the concept of the Holliday junction to create elementary designer nanostructures.[10,11] Seeman recognized that naturally occurring mobile Holliday junctions have strands that are symmetrical, which can lead to the issue of branch migration. Branch migration is the tendency of the branch point of the junction to move along the branch strand.[9] This would lead to insufficient rigidity when creating larger structures. To create rigid 2D and 3D nanostructures Seeman needed to convert Holliday junctions from being transient to having a more permanent shape. He proposed the immobile Holliday junction, which relied on selecting strands that were asymmetric about the junction. The asymmetrical junctions were artificially created to lock the DNA strands in position, thereby preventing branch migration.[11]

To combine immobile Holliday junctions to form nanostructures, Seeman used sticky ends. As discussed earlier, when a portion of the end of a double-stranded DNA helix strand is left as an uncombined single strand (known as an overhang), it will have an affinity for the complementary base pair sequence of another single stranded DNA.[12] When a certain Holliday junction has an overhang, it can bind to another Holliday junction with the complementary base pair sequence. Applying this method to several Holliday junctions facilitated the construction of basic nanoscale tiles.

However, one of the problems of using DNA branched motifs held together by sticky ends was a lack of sufficient mechanical rigidity to create periodic arrays and nanostructures. This was a significant stumbling block in the late 1980s and early 1990s for scientists in the field. A breakthrough was made when it was recognized that junctions could be reinforced with the addition of further helices. This involved making a hybrid of an immobile Holliday junction (J) and a double-crossover (DX) molecule. Double crossovers can form where two helical domains make contact, known as the crossover point.[13]

(Figure 3) This DX+J hybrid structure provided the necessary stiffness required to create robust branched DNA motifs. Seeman later stated that the discovery of the DX molecule as a rigid motif was a very significant leap forward in the field of DNA nanotechnology.[14]

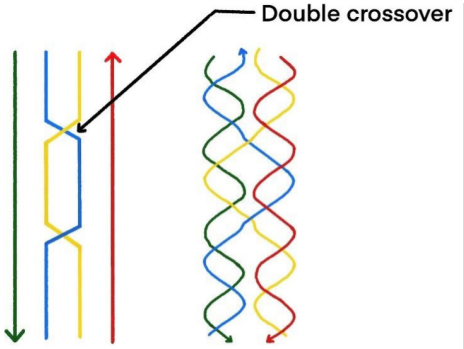

Figure 3: Diagram of a double-crossover (DX) motif on the left. Reinforcing the Holliday junctions with the DX motif provided stiffness to branched DNA structures.

The DX+J hybrid structure was used by Seeman in the self-assembly of DNA to create 2D arrays and crystals.[15] Seeman later also constructed the first 3D closed polyhedral object, a cube-like structure with six faces and eight vertices that corresponded to the branch points of junctions.[16]

Scientists subsequently began to experiment with a range of motifs and tested their rigidity. This included paranemic crossover (PX) motifs as another means of combining DNA. Paranemic crossovers are like double crossovers in that they too form at the crossover point between two helices. However, while double crossovers form only at certain crossover points, paranemic crossovers form at every possible point of contact.[17] With such structural motifs available, scientists have many options when it comes to the combination of DNA.

Single-Stranded DNA Origami:

In the 2000s, scientists took an alternative approach to creating DNA based nanostructures. They built on Seeman's approach of creating structures that form by the process of molecular self-assembly, relying on the specific bonding between DNA base pairs. However, rather than using Holliday junctions and crossover-based hybrid motifs as building blocks, they used single-stranded DNA as the basis for the construction of DNA structures.

The concept of using single-stranded DNA folded into specific shapes using oligonucleotides was pioneered by William Shih and co-workers in 2004.[18] In their method, a 1.7k nucleotide single DNA strand was folded into an octahedron through hybridization with 5 short oligonucleotides. The octahedron has 12 edges, or struts, joined at 6 vertices. The folding of the long scaffold strand was designed to take place in two stages. The first step is when the scaffold and the five associated oligonucleotides assemble to form a "branched-tree" structure. Here, double crossovers between the scaffold and the oligonucleotides form 5 of the 12 struts in the structure. In the second step, the remaining 7 struts are formed as paranemic crossovers, creating the octahedron shape. According to Shih, these two steps are interchangeable, and the order will not affect the formation of the octahedron. Shih's method can be considered the first of its kind in the area of single-stranded DNA origami.

Just two years later, Paul Rothemund introduced his scaffold method,[19] in which a single DNA strand is taken and folded into the desired shape using crossovers facilitated by several smaller oligonucleotides. Rothemund's method differs from Shih's as he uses a single, long 7.2 kilobase M13mp18 scaffold strand. This strand is a naturally occurring bacteriophage, whereas Shih's main scaffold was artificially created. The base pair sequence of the M13mp18 strand is known and fixed, meaning that Rothemund's method could be more easily replicated.

To fold the scaffold strand, Rothemund employed the use of 200 shorter oligonucleotides, or staple strands, as he called them. The design process begins by selecting several double-helices and shortening them sequentially to fit the constraints of the shape being designed, as shown below. To maintain the structure of the double-helices, several crossovers are used between them. Next, the scaffold strand is routed through the double helices such that it, at any point, is part of one of the two strands of the cylinder.[20] (Figure 4)

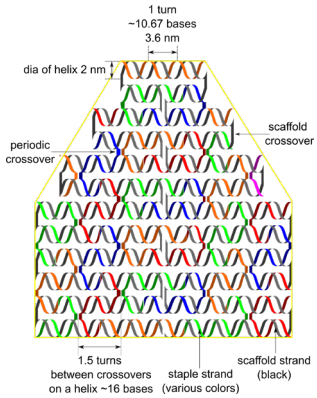

Figure 4: The process of designing a shape using the scaffold method. The double-helices are cut sequentially in order to fit into the constraints of the desired shape. Periodic crossovers are used to maintain the structure of the shape. This image is the author's helical adaptation of an image from Paul Rothemund.

In order to create the DNA origami structures, the scaffold and staple strands are folded using one-pot annealing i.e., the mixture is rapidly heated to near boiling point, and then allowed to cool slowly to allow for self-assembly. The length of this annealing process is dependent on the complexity of the desired DNA origami shape. The structures are then purified by separating out properly formed structures from other molecules, based on their molecular weight. This is typically done using a technique known as agarose gel electrophoresis. The resultant nanostructures are then observed using a microscope with sufficient resolution, such as transmission electron, atomic force and super-resolution optical microscopes. Upon mixing, heating and cooling the mixture of scaffold and staple strands, well-formed structures with yields greater than 90% can be observed under a microscope. Rothemund used his scaffold method to create highly well-known shapes such as a smiley face, and a map of North America.[19] Rothemund's scaffold method truly made DNA origami more accessible to the scientific world.

Tile-Based DNA Origami:

While Rothemund's scaffold method is easily replicable as it involves folding a specific DNA strand (M13mp18 bacteriophage), it does have its limitations. One limitation is that the size of the structures that can be created with it is fixed. The M13mp18 strand is 7,200 nucleotides in length, which corresponds to the construction of origami structures no more than 100 nanometers in diameter.[21] This limited size of DNA origami structures is not an inherent feature of all DNA origami. It is strongly dependent on the shape and scaffold strand chosen. Additionally, the construction of a new shape requires a brand-new set of staple strands to be synthesized.

In 2012, Peng Yin and co-workers introduced the Single Strand Tile (SST) method, using a molecular canvas.[22] While Nadrian Seeman's original work with Holliday junctions could be considered a part of the tile method, modern methods to create tile-based structures take a different approach. In the SST tile approach, each tile or pixel is a unique, 42-base single-strand with four binding domains. In double stranded DNA, 42 bases correspond to four turns of the helix. During self-assembly, an SST tile can bind to four of its neighbors through hybridization, resulting in a lattice consisting of parallel DNA helices that are connected by single stranded linkages (Figures 5a and b). If a certain pixel is not desired to be a part of the final shape, binding is prevented by using edge protectors on its domains. The edge protectors have a sequence complementary to the uncombined binding domain, preventing further combination between certain pixels.

The rectangular lattice of SST tiles is equivalent to a molecular canvas consisting of pixels, each of which can either be included or left out of the final desired shape. The edge protectors are used selectively to exclude certain pixels by preventing them from binding with their neighbors. The strands are held at a temperature above the melting point of the DNA to allow all the DNA strands to separate, followed by a cooling protocol during which they self-assemble. The pixels that are not part of the intended design drop out of the

DOI: 10.36838/v4i3.18

structure, leaving a 2D rendition of the chosen shape. (Figure 5c) The single-stranded tile method allows for a wide variety of shapes to be created. This rationale has been extended by Yin and co-workers into 3D, where they have made shapes as complex as teddy bears and hourglass figures.[23]

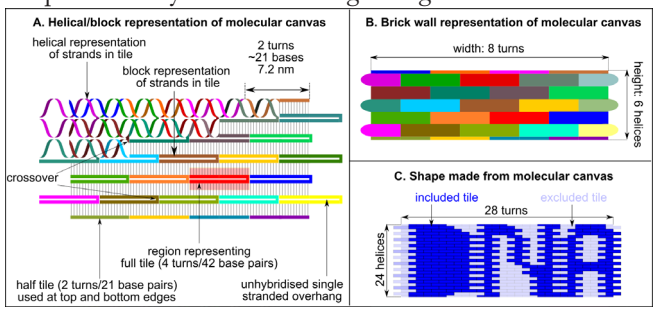

Figure 5: a. Helical and block representation of the molecular canvas formation. Several parallel helices are bound together by crossovers. **b.** The molecular canvas shown in (a) is represented as bricks in a wall, which is then used to create basic shapes. **c.** A shape constructed using the molecular canvas. Excluded tiles (light blue) drop out of the canvas with the use of edge protectors, while the remaining tiles (dark blue) form the shape.

The SST method addresses the limitations of Rothemund's approach by reducing the dependence on a single long strand and making assembly more efficient by using shorter single strands that self-assemble into a range of different shapes. Another intriguing aspect of this method is the surprisingly reasonable yields of successfully formed structures, in the range of ~40%. Researchers had previously concluded that this approach would result in far lower yields, in the range of 20%. Scientists initially anticipated lower yields since it was expected that this method, where concentrations of tile strands are required in perfect stoichiometric proportions, would lead to most structures being poorly formed.[24] The high yields observed mean that Yin's method offers another highly promising way of creating DNA origami.

Yin's single-stranded tile approach is not the only method of creating larger structures via DNA tiles. Lulu Qian and co-workers introduced a method termed "fractal assembly", which combines basic principles from both the single strand scaffold and tile methods.[25] The authors used a scaffold strand along with several staple strands to create nanoscale DNA squares, the surfaces of which have specific patterns. The squares are joined together in a multi-step process, in which squares bind to their neighbors through DNA mediated edge interactions (Figure 6).

The step prior to fractal assembly is synthesis of the individual squares. Each square is formed by a circular scaffold strand as well as edge, interior and bridge staple strands. To create an n x n array requires n^2 test tubes of distinct tiles. In the first stage, test tubes are mixed in groups of four, resulting in the formation of 2 x 2 arrays. At each subsequent step, a larger square array is created from four test tubes of smaller square arrays, that are themselves the product of the immediately preceding stage. The final product thus takes $\log_2 n$ steps.

At any given stage it is necessary to ensure that the corresponding heating cycle does not cause the disassembly of previously created tiles. Qian and co-workers designed the binding edges such that edges that bind in the first step involve the highest number of binding domains and therefore the highest melting points. The melting points of the binding edges and therefore the temperatures required for assembly are designed to systematically decrease with each stage (Figure 6 right panel). This ensures that the binding that occurred at higher temperatures is not reversed. Using fractal assembly, the authors were able to create realistic depictions of complex images, such as that of a rooster and even the Mona Lisa.

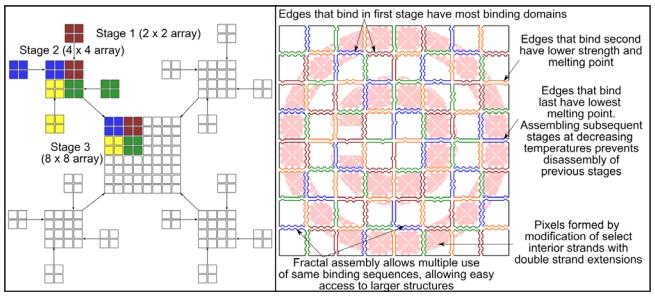

Figure 6: (Left panel) Basic 2x2 arrays combine to form larger 4x4 arrays. This process is repeated recursively $\log_2(n)$ times until the desired n x n array shape is reached. (Right panel) This assembly process can be used to form complex images such as the 'copy left' symbol depicted. The edges are designed such that edges that bind in earlier steps have greater binding strengths and melting points than those in later steps. This ensures that squares formed at higher temperatures in earlier stages do not disassemble.

DNA Origami with Computer Aided Design (CAD):

Since Rothemund's introduction of his scaffold method in 2006, scientists have been making continual progress on designing 2D and 3D nanoscale DNA origami structures. In 2009, Douglas and co-workers introduced CaDNAno[2], an open-source software package intended to streamline the cumbersome process of designing 3D scaffolded structures. CaDNAno supports designs that are created by arranging double helices, either on a honeycomb or square lattice. CaDNAno adapted Rothemund's scaffold method to design 3D shapes formed as layers of double helices.

The process of designing a 3D DNA origami shape in CaDNAno begins by creating the model of the intended shape using cylindrical double-helices that are 2 nm in diameter. The cylinders are required to fit into the constraints of the shape being designed. This is done by cutting the cylinders sequentially, while ensuring that their length corresponds to the helical pitch of DNA i.e., the length should be a multiple of 3.6 nm. A departure from lengths that are multiples of 3.6 nm may lead to a structural failure in the shape. The cylinders that now form the basic structure are held together by crossovers. Once the arrangement of cylinders is finalized, the scaffold strand is routed through the cylinders. In order to fold the scaffold strand, the use of smaller staple strands is employed. The staple strands bind to select points of the scaffold, creating crossover points to fix the cylinders into place.[20] (Figure 7)

The process is completed by the addition of the M13mp18 single strand, the naturally occurring bacteriophage used in Rothemund's scaffold method. Given that the base pair sequence of the M13 is known, it serves as the mechanism for the synthesis of the staple strands, as the staple strands need a

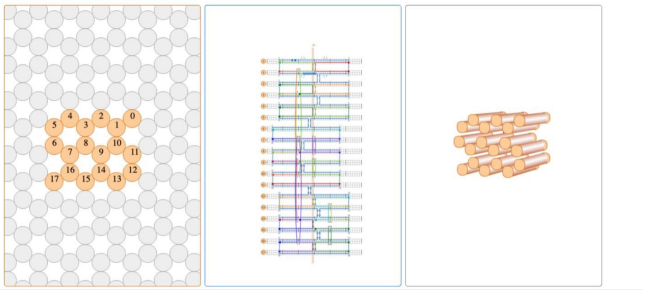

Figure 7: A completed shape designed in CaDNAno. Following the selection of several double helices on the left, users must design a continuous scaffold strand with several staple strands and crossovers to form the desired shape.

specific base pair sequence to be able to bind to select points on the scaffold. One the scaffold strands has been selected and the staple strands have been synthesized, a solution of the two that has been annealed should result in structures that can be observed under a microscope.

While CaDNAno has a simple, intuitive interface and is the most widely used software for DNA origami construction, it has a few limitations. The structures that can be designed using CaDNAno are limited to parallel double-helices structures on either a honeycomb or square lattice. Structures such as 3D tiles, for example, cannot be designed on CaDNAno as they cannot be constrained to this lattice. As the size and complexity of DNA nanotechnology grows, the software that is used to design the structures needs to match it.

To address this, de Llano and co-workers introduced Adenita[3] in 2020, a software tool to design 3D structures including but not limited to the ones that can be designed with CaDNAno. Adenita has the capacity to import designs from CaDNAno, while also creating other structures such as DNA tiles and wireframe structures. This is possible using the Daedalus algorithm, which can convert a 3D DNA origami object into the DNA sequences that are required for its self-assembly.[26] Using the derived sequences, the desired structures can be practically synthesized. Unlike the other software of its kind, Adenita also allows the use of molecules other than DNA (such as proteins) as the building block for the construction of nanoscale objects.

The introduction of software over the past few years such as CaDNAno and Adenita has made DNA origami more accessible to scientists across the field. These tools have streamlined the design process, and the user interface is simple enough that even a high-school student can design basic structures using them!

Applications of DNA Origami:

In its infancy, DNA origami attracted attention due to the creation of unusual shapes or objects using bottom-up self-assembly. However, the field has since progressed well beyond being a mere curiosity or art form. The highly programmable nature of DNA means that it can be used to construct versatile nanoscale structures with practical applications. In the next few years, these structures can have immense use in nanofabrication, medicine, and nano-computation.

In the field of medicine, DNA origami can be used to carry and transport certain molecules, drugs, or proteins. These materials are generally carried on staple strands and given that there can be over 200 staple strands in a standard shape, there is plenty of area for them to be positioned. Zhang and coworkers used this method to deliver doxorubicin, a drug that can treat cancer, to target tumors in mice.[4] Using the M13mp18 scaffold strand along with several staple strands, the authors constructed a DNA origami triangle. The triangle was incubated with doxorubicin for 24 hours, and, following the drug delivery, a fluorescence imaging technique was used to identify the size of the tumor. The mice in which the drug was delivered using DNA origami were found to have tumors significantly smaller than those without the DNA origami delivery over a 12-day period. The primary reason for this is that the double helices that make up the triangle have a significantly large number of binding sites, meaning the drug can be delivered with great specificity to the site of the tumor. Most conventional methods of drug delivery lack this specificity. This experiment demonstrated that DNA origami can have great use in nanofabrication and healthcare.

While several 3D DNA origami structures have been created, from prisms and tetrahedrons to complex octahedrons, prior to 2009, none of these structures had a fully closed surface along with a hollow cavity capable of carrying molecules or drugs. Kjems and co-workers were able to achieve this when they constructed a hollow, 36 x 36 x 42 nm^3 3D DNA origami box from the M13mp18 DNA single strand.[27] This box could also carry a cargo load and was controllable in that it could open on the insertion of two keys into their respective locks located on the exterior of the box. The locks had specific, 8 nucleotide sequences that corresponded to particular DNA keys. When inserted, the box would open if the sequences matched. This DNA origami box was used to carry and deliver drugs to cancer cells.

However, this novel design lacked the ability to repeatedly open and close. In 2012 the Kjems group introduced another iteration of their 3D DNA origami box.[28] Here, the box was switchable, meaning it could open and close several times, and it was 1/7th the size of the original box.

Given the highly controllable manner in which DNA can be folded into desired structures, DNA origami has also been experimented with as a way to inhibit viruses. This is done through the targeted binding of the DNA origami structural motifs to the exterior proteins present on viruses. Wang and co-workers began experimenting with this method in early 2020, when they designed a star-shaped DNA origami structure through tile self-assembly.[6] The ten vertices of the star were formed by a hair loop-like bend of a single strand. This gave the star the structural flexibility necessary to bind to the desired virus under various physical conditions. The vertices of the star also served as the location for ED3-targeting aptamers (single strands capable of folding into specific shapes to bind to targets). The ED3 structure is found on the surface of the dengue (DENV) virus, which the star was designed to inhibit. This DNA-based construction was able to bind to the ED3 structure with high specificity.

The DNA origami star was designed such that when binding between the star and virus took place, it would cause a

DOI: 10.36838/v4i3.18

change in the external environment of dye molecules attached to it. This would result in a fluorescence signal that could easily be detected, indicating that binding between the star and virus was complete. Wang and co-workers' method has begun to be used commercially by Atom Bioworks[29], a firm that maps the arrangement of surface proteins of viruses for use in therapeutics and medicine. In light of the global medical and economic consequences of COVID-19, the application of DNA origami to detecting and inhibiting viruses is likely to attract significant research and commercial interest.[30]

Another area in which DNA origami has seen commercial development is optics and microscopy. Gattaquant[31], a biotechnology company based in Germany, has been one of the first companies to begin using DNA origami for commercial use. Gattaquant uses DNA origami to create several nanoscale structures, from nanorulers to molecular breadboards. The company offers customers these structures for use in super-resolution microscopy, which helps in positioning molecules in desired locations and orientations. Gattaquant's nanorulers can be used to check the resolution of images, and have the ability to serve as a tool to align microscopes.

Discussion and Conclusion

As seen in this review, DNA origami has progressed significantly since its roots in early DNA nanofabrication done in the 1980s. Nadrian Seeman provided the early significant breakthroughs, by recognizing that immobile Holliday junctions could form a basic structural unit, particularly when its rigidity was augmented by double and paranemic crossovers. An important advance in the early part of this century was the use of single stranded DNA scaffolds in combination with smaller staple strands to design nanostructures. More advanced tile-based methods then emerged that addressed some of the drawbacks of using a long single strand scaffold. Recent research work has focused on combining elements of the scaffold and tile-based approaches. All these design efforts have been significantly transformed with the launch of software design packages for DNA based 3D nanostructure modeling. In aggregate, these advances represent rapid scientific progress in an exciting field with significant real-world applications.

Looking ahead, research in DNA origami and nanofabrication is likely to develop in two broad directions. The first is the refinement of DNA origami techniques themselves, to find methods with higher replicability, cost efficiency and yields. The second is research that can support the transition of DNA origami from its theoretical applications in a lab to commercial use in areas like medicine, optics and computing. If advances in both these directions are sustained, DNA origami has a very promising future.

References

1. Seeman, N. C. DNA in a Material World. *Nature* 2003, 421 (6921), 427–431. DOI: 10.1038/nature01406
2. Douglas, S. M.; Marblestone, A. H.; Teerapittayanon, S.; Vazquez, A.; Church, G. M.; Shih, W. M. Rapid Prototyping of 3D DNA-Origami Shapes with caDNAno. *Nucleic Acids Research* 2009, 37 (15), 5001–5006. DOI: 10.1093/nar/gkp436.
3. de Llano, E.; Miao, H.; Ahmadi, Y.; Wilson, A. J.; Beeby, M.; Viola, I.; Barisic, I. Adenita: Interactive 3D Modelling and Visualization of DNA Nanostructures. *Nucleic Acids Research* 2020, 48 (15), 8269–8275. DOI: 10.1093/nar/gkaa593.
4. Zhang, Q.; Jiang, Q.; Li, N.; Dai, L.; Liu, Q.; Song, L.; Wang, J.; Li, Y.; Tian, J.; Ding, B.; Du, Y. DNA Origami as an in VIVO Drug Delivery Vehicle for Cancer Therapy. *ACS Nano* 2014, 8 (7), 6633–6643. DOI: 10.1021/nn502058j.
5. Acuna, G. P.; Möller, F. M.; Holzmeister, P.; Beater, S.; Lalkens, B.; Tinnefeld, P. Fluorescence Enhancement at Docking Sites of DNA-Directed Self-Assembled Nanoantennas. *Science* 2012, 338 (6106), 506-510. DOI: 10.1126/science.1228638.
6. Kwon, P. S.; Ren, S.; Kwon, S.-J.; Kizer, M. E.; Kuo, L.; Xie, M.; Zhu, D.; Zhou, F.; Zhang, F.; Kim, D.; Fraser, K.; Kramer, L. D.; Seeman, N. C.; Dordick, J. S.; Linhardt, R. J.; Chao, J.; Wang, X. Designer DNA Architecture Offers Precise and Multivalent Spatial Pattern-Recognition for Viral Sensing and Inhibition. *Nature Chemistry* 2019, 12 (1), 26–35. DOI: 10.1038/s41557-019-0369-8.
7. Andersen, E. S.; Dong, M.; Nielsen, M. M.; Jahn, K.; Subramani, R.; Mamdouh, W.; Golas, M. M.; Sander, B.; Stark, H.; Oliveira, C. L.; Pedersen, J. S.; Birkedal, V.; Besenbacher, F.; Gothelf, K. V.; Kjems, J. Self-Assembly of a Nanoscale DNA Box with a Controllable Lid. *Nature* 2009, 459 (7243), 73– 76. DOI: 10.1038/nature07971.
8. Adleman, L. M. Molecular Computation of Solutions to Combinatorial Problems. *Science* 1994, 266 (5187), 1021-1024. DOI: 10.1126/science.7973651.
9. Liu, Y.; West, S. C. Happy Hollidays: 40th Anniversary of the Holliday Junction. *Nature Reviews Molecular Cell Biology* 2004, 5 (11), 937–944. DOI: 10.1038/nrm1501. .
10. Seeman, N. C. Nucleic Acid Junctions and Lattices. *Journal of Theoretical Biology* 1982, 99 (2), 237– 247. DOI: 10.1016/0022-5193(82)90002-9.
11. Seeman, N. C. The Design of DNA Sequences for Branched Systems. *In Structural DNA Nanotechnology*; Cambridge University Press: Cambridge, 2015; pp 11–28.
12. Ban, E.; Picu, C. R. Strength of DNA Sticky End Links. *Biomacromolecules* 2013, 15 (1), 143–149. DOI: 10.1021/bm401425k. .
13. Fu, T. J.; Seeman, N. C. DNA Double-Crossover Molecules. *Biochemistry* 1993, 32 (13), 3211–3220. DOI: 10.1021/bi00064a003.
14. Seeman, N. *Structural DNA Nanotechnology*; Cambridge University Press: Cambridge, UK, 2015.
15. Winfree, E.; Liu, F.; Wenzler, L. A.; Seeman, N. C. Design and Self-Assembly of Two-Dimensional DNA Crystals. *Nature* 1998, 394 (6693), 539–544. DOI: 10.1038/28998.
16. Chen, J.; Seeman, N. C. Synthesis from DNA of a Molecule with the Connectivity of a Cube. *Nature* 1991, 350 (6319), 631–633. DOI: 10.1038/350631a0.
17. Shen, Z.; Yan, H.; Wang, T.; Seeman, N. C. Paranemic Crossover DNA: A Generalized Holliday Structure with Applications in Nanotechnology. *Journal of the American Chemical Society* 2004, 126 (6), 1666–1674. DOI: 10.1021/ja038381e.
18. Shih, W. M.; Quispe, J. D.; Joyce, G. F. A 1.7-Kilobase Single-Stranded DNA That Folds into a Nanoscale Octahedron. *Nature* 2004, 427 (6975), 618–621. DOI: 10.1038/nature02307.
19. Rothemund, P. W. K. Folding DNA to Create Nanoscale Shapes and Patterns. *Nature* 2006, 440 (7082), 297–302. DOI: 10.1038/nature04586.
20. P. W. K. Rothemund, Design of DNA origami, ICCAD-2005. IEEE/ACM International Conference on Computer-Aided Design, San Jose, CA, 2005, pp. 471-478, DOI: 10.1109/ICCAD.2005.1560114.
21. Zhang, F.; Yan, H. DNA Self-Assembly Scaled Up. *Nature* 2017,

22.Wei, B.; Dai, M.; Yin, P. Complex Shapes Self-Assembled from Single-Stranded DNA Tiles. *Nature* 2012, 485 (7400), 623–626. DOI: 10.1038/nature11075.
23.Ong, L. L.; Hanikel, N.; Yaghi, O. K.; Grun, C.; Strauss, M. T.; Bron, P.; Lai-Kee-Him, J.; Schueder, F.; Wang, B.; Wang, P.; Kishi, J. Y.; Myhrvold, C.; Zhu, A.; Jungmann, R.; Bellot, G.; Ke, Y.; Yin, P. Programmable Self-Assembly of Three-Dimensional Nanostructures from 10,000 Unique Components. *Nature* 2017, 552 (7683), 72–77. DOI: 10.1038/nature24648.
24.Rothemund, P. W.; Andersen, E. S. The Importance of Being Modular. *Nature* 2012, 485 (7400), 584– 585. DOI: 10.1038/485584a.
25.Tikhomirov, G.; Petersen, P.; Qian, L. Fractal Assembly of Micrometre-Scale DNA Origami Arrays with Arbitrary Patterns. *Nature* 2017, 552 (7683), 67–71. DOI: 10.1038/nature24655.
26.Veneziano, R.; Ratanalert, S.; Zhang, K.; Zhang, F.; Yan, H.; Chiu, W.; Bathe, M. Designer Nanoscale DNA Assemblies Programmed from the Top Down. *Science* 2016, 352 (6293), 1534–1534. DOI: 10.1126/science.aaf4388.
27.Andersen, E. S.; Dong, M.; Nielsen, M. M.; Jahn, K.; Subramani, R.; Mamdouh, W.; Golas, M. M.; Sander, B.; Stark, H.; Oliveira, C. L.; Pedersen, J. S.; Birkedal, V.; Besenbacher, F.; Gothelf, K. V.; Kjems, J. Self-Assembly of a Nanoscale DNA Box with a Controllable Lid. *Nature* 2009, 459 (7243), 73– 76. DOI: 10.1038/nature07971.
28.Zadegan, R. M.; Jepsen, M. D.; Thomsen, K. E.; Okholm, A. H.; Schaffert, D. H.; Andersen, E. S.; Birkedal, V.; Kjems, J. Construction of a 4 Zeptoliters Switchable 3D DNA Box Origami. ACS *Nano* 2012, 6 (11), 10050–10053. DOI: 10.1021/nn303767b.
29.A new platform for antiviral precision medicine. https://atombioworks.com/ (accessed Aug 8, 2021).
30.Sigl, C.; Willner, E. M.; Engelen, W.; Kretzmann, J. A.; Sachenbacher, K.; Liedl, A.; Kolbe, F.; Wilsch, F.; Aghvami, S. A.; Protzer, U.; Hagan, M. F.; Fraden, S.; Dietz, H. Programmable Icosahedral Shell System for Virus Trapping. *Nature Materials* 2021, 20 (9), 1281–1289. DOI: 10.1038/s41563-021-01020-4.
31.Gattaquant. https://www.gattaquant.com/ (accessed Aug 6, 2021).

Author

Kavi Ullal is a student at UWC-USA High School. He has always been passionate about biochemistry and would like to pursue it in his later career. Kavi has been involved in projects in his local community to solve issues pertaining to the availability of natural resources.

DOI: 10.36838/v4i3.18