Design Techniques for the DNA Cubic-Lattice

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ABSTRACT

We use the Watson-Crick properties of DNA and the principles of graph theory to construct origami folding designs for self-assembling cubic lattices. Our objective is a mathematical design strategy that can be expanded systematically to any size cubic lattice. This design consists of threading a scaffolding strand of DNA through the lattice that is secured in place by short staple strands of DNA. We first add augmenting edges to the cubic lattice to enable a single scaffolding strand threading. We then thread the scaffolding strand through the augmented cube in a way that minimizes the number of different vertex configurations in the structure.

KEYWORDS

Watson-Crick, DNA Self-Assembly, Origami folding, Cubic Lattice, Scaffolding Strand, Threading, Staple Strands

INTRODUCTION

Recent research has focused on the self-assembly of DNA structures, which can be used for biomolecular computing, targeted drug delivery and biosensors.¹ There are several structures that have already been created from self-assembling DNA molecules, such as cubes,² truncated octahedra,³ rigid octahedra,⁴ tetrahedra, and dodecahedra.⁵ The construction methods of these nanostructures have evolved over time. The branch junction molecule method, or tile based method, previously dominated the field, but as time has progressed, so have the methods of DNA construction. Origami methods have started to become the foremost techniques in the self-assembly of DNA.¹ This method begins with a single scaffolding strand of DNA that traces the edges of the structure exactly once, and shorter strands bond to this strand to fold and staple it into the desired configuration. The origami method has been specifically used in the construction of nanorobots and carriers, drug and material delivery and directed positioning of particles and materials.¹

In this paper, we will be working with a design for the cubic lattice. We begin with a description of the design and threading of a 1x1x1 cube. From this we gain insight into cubic lattices of all sizes noting that the first step must be to add augmenting edges so that all vertices have an even degree. We provide three different augmenting strategies, two for $n \times n \times n$ cubes with n odd and one for n even. We then give a complete threading and stapling of the augmented 2x2x2 and 3x3x3 cubes.

DNA BACKGROUND

Deoxyribonucleic acid (DNA) is typically in a double helix form that is constructed from two separate strands of DNA that are complements to one another. Each strand consists of a backbone made up of sugar and phosphate molecules and nitrogenous bases that hold the information stored in the DNA. The four bases are: Adenine (A), thymine (T), guanine (G) and cytosine (C). These bases pair off with one another to form base pairs that form the treads of the DNA double helix; A must pair with T, and C must pair with G. In order for a strand of DNA to bond with another, the sequences of bases must be perfectly complementary to one another. For example, a strand GTACCTA can only bond with its opposite strand, CATGGAT. When placed in the right solution, the self-assembly properties in DNA will allow a strand to bond together with its complementary bases and form a double strand.⁶

To create the cubic lattice using the method of origami folding, we want to take a single strand of DNA and wrap it around every edge of the structure exactly once. The precise sequence of bases in this strand is either custom designed or comes from an existing virus strand, so we do not specify it here. This strand of DNA is called a scaffolding strand, and the process of wrapping the scaffolding strand around the structure is called a threading. DNA is directional, and therefore its direction must also be accounted for in the design of any structure. For this threading to be possible, we need an Eulerian cycle to be present in the lattice. An Euler cycle is a path that runs through a graph and covers each edge exactly once. Vertices may be visited multiple times, but no edges can be repeated, and the cycle must start and end at the same place.⁷ This is only possible if each vertex in the graph is of even degree.⁷ The degree of a vertex is determined by the number of edges that go into and out of the vertex. Once all vertices have even degree, the scaffolding strand of DNA can be threaded throughout the structure.

In order for the structure to remain in the shape of the lattice, we must attach staple strands into the structure to hold the lattice in its shape. These staples are added at the vertices of the structure to ensure the structure remains intact. The staple strands must be perfect complements to the portions of the scaffolding strands that they hold, and they must be directed in the opposite directions of the threading. The following constraints must be satisfied by the threading and stapling processes.

Threading and stapling constraints:

- Every edge must be included in the threading exactly once.
- Each vertex must have the same in-degree as out-degree. In other words, we must have the same number of edges entering a vertex as leaving it.
- We can only thread/staple angles of 45° and 90°, not 180°.
- We cannot cross over any edges while stapling/threading: i.e. a staple or thread should not be forced to leave the plane formed by the two edges it follows by an edge between them.
- Staple strands must be oriented oppositely from the scaffolding strand.

Figure 1. Depiction of prohibited thread and staple configurations. The figure on the left shows a 180° thread and staple configuration, so the structure would “detach” at this vertex. The center figure shows staples that are traveling in the same direction as the threads. The figure on the right shows 5 coplanar edges, and a sixth vertical edge perpendicular to them, with a staple that has crossed over an edge in the same plane as the stapled edges.

LATTICE BACKGROUND

A cubic lattice is a structure that is made up of unit-sized cubes. When we talk about cube sizes, we will refer to a cube as size n . For example, when we discuss a cube that is $3 \times 3 \times 3$, it will be referred to as a size 3 cube. In order to find an Euler cycle in the lattice, we need each vertex to have even degree. In the case of the cubic lattice, the structure must be modified to meet this constraint. Each corner of the lattice has degree three and all of the vertices in the middle of each face not touching any corner have degree five. (Figure 2)

Figure 2. A size 4 cube showing the odd degree vertices on one face of the un-augmented cubic lattice.

In order to solve the problem of odd degree vertices, we have to add augmenting, diagonal edges that turn the corners of the cube into either degree four or degree six vertices, and turn the degree five vertices on the outer faces into vertices of degree either six or eight. We do not have to add augmenting edges to any of the interior vertices since they all have degree six. As we developed design strategies for this structure, we wanted to keep the structure as stable as possible and maintain an easily expandable pattern throughout. To do this, we must add augmenting diagonals to the outside of the cube in a systematic way to preserve the cubes rigidity and symmetry.

PRELIMINARY OBSERVATIONS

- There is a difference between cubes of even parity and those of odd parity. Even cubes (size n , for $n = 2, 4, 6, \dots$) all have a center vertex in the middle of the structure and in the middle of each face. Odd cubes (size n , for $n = 1, 3, 5, \dots$) have a cube in the center of each structure and a square in the middle of each face.
- With this difference between even and odd sized cubes, different design strategies must be considered for each type.

BASIC SIZE 1 CUBIC LATTICE

We started with a single cube to understand the general nature of cubic structures. A cube is made up of six square faces containing twelve edges and eight vertices each with degree three. However, there is no Euler cycle through the cube due to the odd degree of all of the vertices. Therefore, we have to modify the cube to ensure that all vertices have even degree. This can be achieved by adding a diagonal augmenting edge to each one of the four walls of the cube. With this modification made, we now have eight vertices of degree four and 16 edges. The threading of the cube is depicted in Figure 3 with its respective staples at each vertex. When we discuss a cube, we use the 3D Cartesian coordinate system to locate each vertex in the structure. We place a corner of the cube at the origin, $(0,0,0)$, and extend the rest of the structure along the positive x , y and z axes.

Figure 3. Threading and stapling for a size 1 cube with augmenting edges.

When we consider the threading configuration for each vertex, two different vertex configurations emerge. Vertex configurations that are the same can be rotated to fit the cube as long as the threading and stapling patterns do not change. The difference between the two vertex configurations is in the direction and placement of the threading and staple strands. In the case of the basic size 1 cube, the two different vertex types are mirror images of each other, meaning that both thread

a 45° angle and a 90° angle but travel in opposite directions. This makes them different, yet they still remain symmetric. Figure 4(a) and 4(b) show the two types of vertex configurations that are contained in the size 1 cube along with the coordinates that contain those vertex configurations.

Figure 4. Depicts the two different types of vertex configurations in the size 1 cube.

From this cube we have determined that each corner vertex in a cubic lattice will have to have an odd number of diagonals extending from it to make all corner vertices have even degree. More specifically, there must be one or three diagonals due to the fact that there cannot be more than three diagonals protruding from one corner vertex since all corners of a cube are naturally degree three. Therefore, we cannot have an even number of diagonals coming from any of the corners.

GENERAL AUGMENTING EDGE DESIGN OF ODD CUBES

Our designs for each odd size cube, for $n > 1$, have a diagonal in each corner of each face. As the cube increases in dimension, more parallel diagonals are added vertically and horizontally from the corner. For example, the size 3 cube has only one diagonal in each corner. The size 5 cube also has a diagonal in each corner plus a parallel diagonal vertically and horizontally adjacent to the corner diagonal. The number of diagonals in each corner continues to grow in this fashion as the cube expands (see Figure 5).

Figure 5. Depiction of corner diagonal increasing horizontally and vertically as the odd cube expands.

After we placed these diagonals in each corner of each face we realized that we needed to consider two equivalence classes of odd cubes and use different strategies for the two classes. The congruent modulo m relation ($a \equiv b \pmod{m}$), where $m = 4$, is the equivalence relation that we used. This means all integers that have the same remainder after division by m are in the same equivalence class. We found that the odd size cubes, for $n = 3, 7, 11, \dots$, which are congruent to $3 \pmod{4}$, can be constructed in the same manner. Odd size cubes, for $n = 5, 9, 13, \dots$, which are congruent to $1 \pmod{4}$, can be constructed in the same manner (different from the design of odd cubes congruent to $3 \pmod{4}$).

DESCRIPTION OF GENERAL DESIGN FOR CUBES CONGRUENT TO 3 MOD 4

Each face of the odd sized cubes greater than $n = 3$ and congruent to 3 mod 4 ($n = 7, 11, 15, \dots$) begins like all odd size cubes, by adding diagonals along edges as shown in Figure 5. Next, we build small candelabras into the center of the face from each corner (see Figure 6). Candelabras should be added to all corners evenly, so if there are 4 candelabras in one corner then all other corners should also have four candelabras. The candelabras create a square in each corner of each face of the cube. This pattern begins as a single candelabra in the size 7 cube, then becomes a 2x2 arrangement of candelabras in the size 11 cube, a 3x3 arrangement of candelabras in the size 15 cube and continues in this fashion (see figure 7a.). All problem spots are resolved with these candelabras, but in each face is a cross of empty squares, which could cause the cube to shear. To address this, diagonals are added in a “zigzagging” pattern to wrap around four of the faces of the cube (see figure 7b). When the cube is completed there will be four identical faces which will make up the walls, and the two remaining faces will be identical and form the top and bottom of the cube. The only difference between the four identical faces and the two identical faces is the “zigzag” pattern. (See Appendix for figures of odd designs congruent to 3 mod 4)

Figure 6. Depiction of small candelabra used in the designs of odd cubes size 5 and greater.

Figure 7a. Depiction of square formed by candelabras in the corner of each face.

Figure 7b. Depiction of “zigzag” pattern that stabilizes the cubes with size congruent to 3 mod 4.

DESCRIPTION OF GENERAL DESIGN FOR ODD CUBES CONGRUENT TO 1 MOD 4

Each face of the odd sized cubes congruent to 1 mod 4 ($n = 5, 9, 13, \dots$) is created with the same small candelabras (see Figure 6). The design for each face of these cubes begins with the placement of diagonals in the corners and along edges as described above and shown in Figure 5. The candelabras in these cubes are added to each corner in each face in a pinwheel fashion. Instead of creating squares of candelabras in each corner, this design creates rectangles of candelabras that rotate in each corner. The rectangle begins as a 1x2 arrangement of candelabras in the size 9 cube, then a 2x3 arrangement in the size 13 cube, a 3x4 arrangement in the size 17 cube, and continues in this fashion (see Figure 8a). To complete the design, a single candelabrum is placed off of one of the rectangles formed in the corners. When looking at a T-Diagram of the cube (see the Appendix for T-Diagram definition), we have placed this candelabra off of the upper left rectangle of candelabras in each face (see Figure 8b). However, there are multiple ways to place this last candelabrum in each face. The placement we chose is purely for simplicity in constructing the cube. The size 5 cube is too small to include the pin-wheeling candelabras, so each face only contains the diagonals and the one candelabra coming off the upper left corner (see Appendix for Figures of odd designs congruent to 1 mod 4).

Figure 8a. Depiction of pin-wheeling rectangle formed by cubes congruent to 1 mod 4.

Figure 8b. Depiction of center candelabra used in cubes congruent to 1 mod 4.

SPECIFIC SIZE 3 CUBE DESIGN, THREADING AND STAPLING

The original size 3 cube contains 64 vertices and 144 edges, and with this original configuration, there are 32 vertices of odd degree. Since this is an odd sized cube, we placed diagonals branching out of each corner of each face. This fixes all 32 of the odd degree vertices by turning the corners from degree three vertices to degree six vertices and turning all of the degree five vertices into vertices of degree six. Since the corner diagonals fix all of our odd degree vertices, we do not need to add any candelabras to the structure. However, since the size 3 cube falls under the category of cubes that are congruent to 3 mod 4, we must place the “zigzagging” pattern through the middle of the cube to prevent it from shearing. The path starts at vertex $(0,0,1)$, travels up to $(0,1,2)$ and then travels down to $(0,2,1)$. The path continues this “zigzag” pattern all the way around the cube and connects with itself at vertex $(0,0,1)$.

The threading of the size 3 cube was done with a “knitting” like approach. The knitting approach of threading slowly traverses the cube by threading sections of the cube at a time. In our case, we threaded the cube column by column (see Figure 9). This style of threading is different than a weaving approach that continuously threads itself from one corner of the cube all the way to the opposite corner and back again. In order for the threading to be possible, we need to ensure that there is an Euler cycle present in the structure that follows the angle constraints of 45 and 90 degrees respectively. Our goal is to create a threading that has symmetry and a fairly simple pattern that can be repeated for other cube sizes.

1	2	3
8	9	4
7	6	5

Figure 9. A labeling of columns in the size 3 cube

The threading starts at $(0,0,0)$ and travels up the first column and down the second, back up the third and down the fourth, etc. (See Appendix for complete threading sequence). Once the cube is completely threaded in this manner, we are able to see that the structure has symmetry in opposite columns. Columns one and five are almost perfectly symmetric (with the exception of one vertex), and columns three and seven are perfectly symmetric. In all, there are 23 different vertex threading configurations in this structure with 64 total vertices (see Figures 10 through 12).

Figure 10. Vertices exclusively contained in columns 1 and 5,

Figure 11. Vertices exclusively contained in columns 3 & 7.

Figure 12. Vertices that are contained in all four columns 1,3,5 & 7

GENERAL AUGMENTING EDGE DESIGN OF EVEN CUBES

Unlike the odd sized cubes which have a square in the middle of each face and a cube at the center of its structure, the even sized cubes ($n = 2, 4, 6, \dots$), as discussed in the preliminary observations, have a vertex in the center of each face and in the very center of the entire structure. This makes a difference in approaching possible designs, because there are an odd number of vertices inside each face of the even cube and an even number of vertices per face for odd cubes. The odd number of vertices in each face of an even sized cube makes it impossible to pair all vertices in one face. However, since there is an even number of vertices on the surface of the entire cube, we can pair an odd degree vertex on one face with an odd degree vertex on an adjacent face and make them even. Our general even design is created by building large candelabras into the center from two opposite corners (see Figure 13a). These large candelabras are built in towards the middle until the ends of the candelabras from opposite corners connect. At the vertex where the ends of the candelabras connect, a diagonal is added to create a "T" (see Figure 13b). We omit an edge from one candelabra in order to create one odd degree vertex that can then pair with the odd degree vertex on an adjacent face (see Figure 14 for construction of the even cube face). The design of one face of the cube can be rotated in different directions to create the faces of the entire cube. The completed cube will have three pairs of identical faces (see Figure 15 for the proper rotation of faces).

Figure 13. (a) depicts the large candelabra used in the even cube design, and (b) shows the "T" that is created after the opposite candelabras connect.

Figure 14. (a) depicts the construction of large candelabras from one corner of a face for a size 8 cube. (b) Shows the construction of candelabras starting from the opposite corner leaving one unconnected candelabra that connects with its adjacent face. (c) Depicts the additional diagonals that form the “T” shape discussed above.

Figure 15. Depicts the rotation of even cube faces.

SPECIFIC SIZE 2 CUBE DESIGN, THREADING AND STAPLING

The original size 2 cube consists of 27 vertices and 54 edges, and out of these 27 vertices, 12 of them are of odd degree. To make these vertices even degree, augmenting edges are added to make a total of 66 edges. Three diagonals are placed coming out of two corner vertices that are opposite of each other, $(0,0,0)$ and $(2,2,2)$. The rest of the corner vertices only have one diagonal coming out of them. The diagonals should be placed so that the completed cube has two different types of faces (see Figure 16). One type of face contains three diagonals that form the “T” shape used in all even cubes, and the other contains only one diagonal protruding from the corner vertex. These two types can be rotated to create all other faces of the cube (see Appendix for T-diagram). Now, all vertices become a degree 4, 6, or 8, making it possible to thread the cube.

The threading of the size 2 is done in the same knitting like pattern as the size 3 cube and traverses the cube column by column in a similar manner. However, since the cube design itself has less than ideal symmetry, the threading sequence also has less symmetry. Opposite corners, $(0,0,0)$ and $(2,2,2)$, are purposely threaded exactly the same and the rest of the cube’s threading follows from that. The final threading for the size 2 cube has 16 different vertex configurations for 27 vertices (see Figure 17).

Figure 16. (a) and (b) depict the two types of faces used to construct the size 2 cube.

Figure 17. Depicts all vertex configurations in the threading and stapling of the size 2 cube.

CONCLUSION

Through our work with the cubic lattice, we have developed designs that ensure an Euler cycle exists in all of the cubic structures. The designs that have been created are specific to the parity of the cube, odd or even. In the case of the odd cubes, there are two types of equivalence classes that exist, cubes congruent to 1 mod 4 and cubes congruent to 3 mod 4. All of these designs contain relative symmetry and are able to expand. In the specific cases of size 1, 2 and 3 cubes, we provided threading configurations and techniques that can be applied to the construction of these cubes. These threading techniques seem to work well for the cubic lattice; however we wish to further develop a more general threading technique that can be applied to all cubes in the future.

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SUPPLEMENTAL INFORMATION

Supplemental appendices may be found online in PDF format alongside the associated article at www.ajuronline.org.

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PRESS SUMMARY

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