DNA Tile Self-Assembly for 3D-Surfaces: Towards Genus Identification

Florent Becker
Univ. Orléans, INSA Centre Val de Loire, LIFO EA 4022, F-45067 Orléans, France

Shahrzad Heydarshahi
Univ. Orléans, INSA Centre Val de Loire, LIFO EA 4022, F-45067 Orléans, France

Abstract

We introduce a new DNA tile self-assembly model: the Surface Flexible Tile Assembly Model (SFTAM), where 2D tiles are placed on host 3D surfaces made of axis-parallel unit cubes glued together by their faces, called polycubes. The bonds are flexible, so that the assembly can bind on the edges of the polycube. We are interested in the study of SFTAM self-assemblies on 3D surfaces which are not always embeddable in the Euclidean plane, in order to compare their different behaviors and to compute the topological properties of the host surfaces.

We focus on a family of polycubes called *order-1 cuboids*. *Order-0 cuboids* are polycubes that have six rectangular faces, and order-1 cuboids are made from two order-0 cuboids by substracting one from the other. Thus, order-1 cuboids can be of genus 0 or of genus 1 (then they contain a tunnel). We are interested in the genus of these structures, and we present a SFTAM tile assembly system that determines the genus of a given order-1 cuboid. The SFTAM tile assembly system which we design, contains a specific set Y of tile types with the following properties. If the assembly is made on a host order-1 cuboid C of genus 0, no tile of Y appears in any producible assembly, but if C has genus 1, every terminal assembly contains at least one tile of Y.

Thus, for order-1 cuboids our system is able to distinguish the host surfaces according to their genus, by the tiles used in the assembly. This system is specific to order-1 cuboids but we can expect the techniques we use to be generalizable to other families of shapes.

2012 ACM Subject Classification Theory of computation → Models of computation

Keywords and phrases Tile self-assembly, DNA computing, Geometric surfaces

Digital Object Identifier 10.4230/LIPIcs.DNA.29.2

Related Version *Full Version*: https://arxiv.org/abs/2210.13094

Funding Supported by ANR project SyDySi (ANR JCJC 2019 19-CE48-0007-01).

1 Introduction

In this paper, we introduce a new tile self-assembly model in order to perform self-assembly on 3-dimensional surfaces. The field of tile self-assemby originates in the work of Wang [14], who introduced in 1961 *Wang tiles*, that is, equally sized 2-dimensional unit squares with labels/colors on each edge (later called *glues*) and designed a Turing universal computation model based on these tiles. In 1998, inspired by Wang tiles and DNA complexes from Seeman's laboratory [6], Winfree introduced in his PhD thesis [15] the *abstract Tile Assembly Model* (aTAM). This model uses Wang tiling with an extra information: he associated a non-negative integer strength for each glue label. The power of DNA self-assembly enables to compute using this model. We refer to the survey [9] for more details on the literature, and to the online bibliography of Seeman's laboratory [12].

Most of the early work in the DNA tile self-assembly literature deals with rigid assemblies in the Euclidean plane [9, 10] (since the assemblies are discrete, the Euclidean plane is usually seen as the lattice $\mathbb{Z}^2$), which is a natural and simple setting for this model. However, it can be interesting to use self-assembly in richer settings. This has been investigated experimentally for instance in [13, 16, 17] where the assembly takes place on a preexisting surface and changes according to the surface. On the theoretical side, there have been some recent works on DNA tile self-assembly outside the Euclidean plane, such as tile self-assembly in mazes [4], where the tile placement is done on the walls of a certain maze. Other types of self-assembly exist that also do not use the Euclidean plane, for example a model of cross-shaped origami tiles [18]. Another type of self-assembly not in the plane is 3D assemblies of complex molecules like crystals [3, 7]. Inspired by this, a recent model called *Flexible Tile Assembly Model* (FTAM) was introduced by Durand-Lose et al. in 2020 [5], as an extension of earlier work [8]. Here, we have Wang tiles but they self-assemble (without an input surface) in 3D space (modeled by the lattice $\mathbb{Z}^3$) as they can have, in addition to standard *rigid* bonds, *flexible* bonds that allow tiles to bind at any angle along the tile edges. The goal of the FTAM model is to construct complex 3D structures called *polycubes* (3D shapes made of unit cubes) [2].

In 2010, Abel et al. [1] used a variant of the aTAM to implement shape replication, where tiles react to the shape of a preexisting pattern to reproduce it. The assembly takes place on the 1-dimensional border of a 2D pattern. Here, the main challenge is that the system must react to the shape of the space around, rather than to an external input it can read as it wants.

We are interested in studying what happens if we put the tiles on a given 3D surface, that is not necessarily topologically equivalent to the Euclidean plane. The intuition is that this could modify the computational behaviour of the tile self-assembly model, and we believe it will be interesting for practical systems, as in some practical settings, self-assembly could be performed on complex surfaces.

Inspired by the FTAM, we introduce a new model, called *Surface Flexible Tile Assembly Model* (SFTAM). In the SFTAM, we are given a 3D surface, on which the tiles of the self-assembly get placed. The SFTAM is an intermediate between aTAM and FTAM. Unlike in the FTAM, our aim for introducing the SFTAM is *not* for building 3D structures or surfaces: we assume that the host surface already exists. In the SFTAM, tile bonds are all flexible and the tiles can bind along the edges of the surface.

This model enables to use self-assembly on surfaces other than $\mathbb{Z}^2$. The aim of this article is to introduce the SFTAM model, and to demonstrate its usefulness by showing how it can be used on various types of surfaces. One of the most important properties of a surface is its *genus*, which, intuitively, is the number of "holes" in the surface. The Euclidean plane has genus 0. We are interested in using the SFTAM on surfaces with different values of genus. For that, we study the problem of characterizing the surface of the assembly, according to its genus, using the SFTAM. It is quit easy to devise a system which can behave in some way only on the torus, but it is harder to make sure that it has always this behavior when it is in fact on a torus.

We focus on a family of 3D surfaces called *cuboids*, which are special types of polycubes. Polycubes can form complex surfaces, and their genus can be high. We focus on a simple family of polycubes that can have genus 0 or genus 1. More specifically, we define an *order-0 cuboid* as a polycube which has only six faces. An *order-1 cuboid* $C_1 = C_0 \setminus C'_0$ is a polycube that is made from the difference of two order-0 cuboids C_0 and C'_0. Thus, an order-0 cuboid is a simple surface with genus 0, but an order-1 cuboid can either have genus 0 (potentially with a pit or concavity) or genus 1, if it has a tunnel.

In this paper, we will suppose that the SFTAM self-assembly is performed on the surface of an order-1 cuboid C. We design an SFTAM system whose assemblies differ when C is of genus 0 and of genus 1 and thus, can be used to detect the genus of the surface C of the

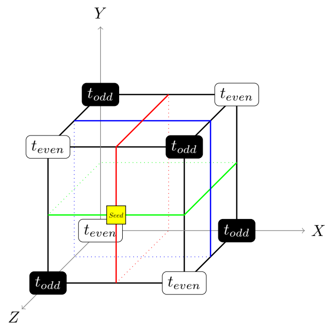


Figure 1 The skeleton of a $\mathcal{S}_{\mathcal{G}}$ assembly on an order-0 cuboid is shown in color. It is started from a seed (in yellow) and after the formation of the skeleton, the regions are partially filled by tiles of types t_{odd} and t_{even}.

assembly it is used on. The goal of this study is to show that performing self-assembly on surfaces of higher genus can be helpful. We also demonstrate some techniques which may prove useful in characterizing the topological properties of a wide range of surfaces.

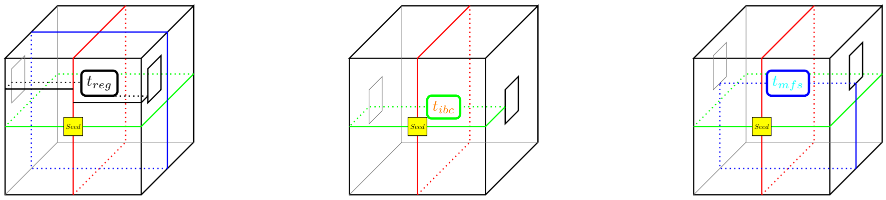
A *tile assembly system* (TAS) in the SFTAM is defined in a natural way as an extension of the aTAM: tile types are made of four glue labels, each has a strength, there is a seed assembly and a temperature (more formal definitions will be given later). An *assembly* is a placement of tiles on facets of the surface of the cuboid C. Two tiles bind if they are adjacent (i.e. their placements on the surface share an edge) and their glue labels are the same. In particular, edges are flexible and as a result the tiles can be placed on the border of orthogonal faces of C. The assembly is *producible* if it can be obtained by successfully binding tiles, starting from a seed . It is *terminal* if no additional tile can be bound to an existing tile.

Let $C = C_0 \setminus C_0'$ be an order-1 cuboid with its three dimensions at least 10 for C_0'. Our main result is to describe an SFTAM (TAS) $\mathcal{S}_{\mathcal{G}}$ with a subset Y of its tile types such that the following holds:

- if the order-1 cuboid C has genus 0, then no tile of Y appears in any producible assembly of $\mathcal{S}_{\mathcal{G}}$ on C, and
- if C has genus 1, every terminal assembly of $\mathcal{S}_{\mathcal{G}}$ on C contains at least one tile of Y.

In other words, the genus of C can be determined using the assemblies of $\mathcal{S}_{\mathcal{G}}$ on C. The assemblies of $\mathcal{S}_{\mathcal{G}}$ consist of two phases: a skeleton forms on the cuboid and separates it into several regions, then the regions are partially filled by inner tiles. See Fig. 1 for a sketch of the skeleton and its inner filling for an order-1 cuboid with genus 0. When the cuboid has genus 1, we show that there must be some parts of the skeleton or the inner filling which intersect in a way that is not possible on a genus-0 cuboid. The tile types of Y stick at the place where this happens. See Fig. 2.

We start with basic definitions and notations in Section 2, where we introduce and formalize our SFTAM model. Next, we introduce the family of order-1 cuboids and we show how the SFTAM behaves on the family of order-1 cuboids as an assembly model in three dimensions. In Section 3 we develop technical lemmas that will be necessary for the proof of our main result. In Section 4 we present our main result: a SFTAM tile assembly system that identifies the genus of order-1 cuboids using specific tiles from that system. We conclude in Section 5. Due to space constraints, some parts of the proofs and details are deferred to the appendix.



(a) The case where the skeleton does not meet the tunnel. In this case, tiles of types t_{odd} and t_{even} located in the two regions containing the entrances of the tunnel, pass inside the tunnel. Where they meet, a tile of type t_{reg} appears in the assembly.

(b) The tunnel intersects along the width of plane P_X and length of plane P_Y. The green tile is of type t_{ibc} (a tile type from T_{ibc}).

(c) The case where the tunnel of an order-1 cuboid is shown by a tile of type t_{mfs}, located at the intersection of the skeleton.

Figure 2 According to the relative position of the seed and the tunnel, the detection of the tunnel is done by different tile types of $\mathcal{S}_{\mathcal{G}}$. The seed is indicated in yellow and the skeleton is in color.

2 Definitions and notations

We now define the Surface Flexible Tile assembly Model, SFTAM. We work in 3-dimensional space, on the integer lattice $\mathbb{Z}^3$.

▶ **Definition 1** (Tile type in SFTAM). *Let Σ be a finite label alphabet and ϵ represent the null label. A* tile type *t is a 4-tuple $t = (t_1, t_2, t_3, t_4)$ with $t_i \in \Sigma \cup \{\epsilon\}$ for each $i = \{1, 2, 3, 4\}$. Each copy of a tile type is a* tile *and t_1, t_2, t_3, t_4 are the glues of t.*

Tiles are $2D$ unit squares whose sides are assigned the labels of the tile type. These squares are allowed to translate and rotate (unlike in aTAM), but they can not be mirrored (unlike in FTAM). In fact, since the tiles stick to a given surface, we can assume that they have an inner face and an outer face and that they always attach with the inner face in contact with the surface. In the definition of tile types, we show labels by numbers rather than cardinal directions. However, often, the orientation of a tile dictates the orientation of the tiles around it. Then, we use the expression "northern label" to refer to the label which will end up on the northern side (and similarly for east, west, south).

▶ **Definition 2** (Facet). *A* facet *is a face of the lattice $\mathbb{Z}^3$, i.e. a unit square whose vertices have integer coordinates.*

▶ **Definition 3** (Polycube). *A* polycube *is a 3D structure that is a subset of $\mathbb{Z}^3$ and is formed by the union of unit cubes that are attached by their faces.*

For a facet of a polycube, there are four possibilities for placing a tile.

▶ **Definition 4** (Placement). *Let C be a polycube. A* placement *$p = (f, o)$ on C consists of a facet f on the surface of C, and a side o of f, called its* orientation.

We denote the set of all placements in C by $Pl(C)$.

Given a tile type $t = (t_1, t_2, t_3, t_4)$ and a placement $p = (f, o)$, placing *t at the placement p defines a mapping from the edges of f to the label alphabet Σ. The i-th side of f (starting from the orientation o and going in clockwise direction, looking from the exterior of the surface of C) is associated with t_i.*

▶ **Definition 5** (Tile assembly system (TAS) on a polycube in SFTAM). *A* tile assembly system, *or TAS, over the surface of a given polycube C is a quintuple $\mathcal{S} = (\Sigma, T, \sigma, str, \tau)$, where :*

- *Σ is a finite label alphabet,*

- *T is a finite set of tile types on Σ,*
- *σ is called the* seed *and can be a single tile or several tiles*
- *str is a function from $\Sigma \cup \{\epsilon\}$ to non-negative integers called* strength function *such that $str(\epsilon) = 0$, and*
- *$\tau \in \mathbb{N}$ is called the* temperature.

While the SFTAM is a theoretical system, its components have an analogy with elements of practical DNA settings. The labels are the single strands of DNA, the function *str* show the strength of their connections and the τ is the temperature.

We present the definitions and notations of SFTAM assemblies that we will use throughout the article. They define similar to the ones for the aTAM [9].

▶ **Definition 6.** *An* assembly *α of a SFTAM TAS $\mathcal{S}$ on a polycube C is a partial function $\alpha : \mathrm{Pl(C)} \dashrightarrow T$ defined on at least one placement such that for each facet f of C, there is at most one placement (f, o) where α is defined.*

For placements $p = (f, o)$, $p' = (f', o')$ of $\mathrm{Pl(C)}$ with $\alpha(p) = t$ and $\alpha(p') = t'$ such that f and f' are distinct but have a common side s, we say that t and t' bind *together with the strength st if the glues of t and t' placed on s are equal and have the strength st.*

The assembly graph *G_α associated to α has as its vertices, the placements of $\mathrm{Pl(C)}$ that have an image by α, and two placements p and p' are adjacent in G_α if the tiles $\alpha(p)$ and $\alpha(p')$ bind.*

*An assembly α is τ-*stable *if for breaking G_α to any smaller assemblies, the sum of the strengths of disconnected edges of G_α needs to be at least τ.*

▶ **Definition 7.** *Let C be a polycube and $\mathcal{S} = (\Sigma, T, \sigma, str, \tau)$ a SFTAM TAS with σ positioned on a placement of C. An assembly α of $\mathcal{S}$ is* producible *on C if either $dom(\alpha) = \{p\}$ and $\alpha(p) = \sigma$ where $p \in \mathrm{Pl(C)}$, or if α can be obtained from a producible assembly β by adding a single tile from $T \setminus \sigma$ on C, such that α is $\tau - stable$. Note that $dom(\alpha)$ is the domain of the assembly α. We denote the set of producible assemblies of $\mathcal{S}$ by $A^C[\mathcal{S}]$. An assembly is* terminal *if no tile can be τ-stably attached on C. The set of producible, terminal assemblies of $\mathcal{S}$ is denoted by $A^C_\square[\mathcal{S}]$.*

The SFTAM assemblies start from a seed and growth by a one by one tile adding. A tile can be added to an assembly in any placement on the host surface where it binds to the existing assembly with total strength at least τ with each pair of matching edges contributing the strength of its glue.

We now introduce *order-1 cuboids*, which are special types of polycubes.

▶ **Definition 8** (Order-0 cuboid)**.** *An* order-0 cuboid *$C = (s_C, x_C, y_C, z_C)$ where $s_C = (s_x, s_y, s_z) \in \mathbb{Z}^3$ is the point of C with smallest coordinates and x_C, y_C, z_C are integers representing the length, width and height of C is a 3D structure containing all points (x, y, z) of $\mathbb{Z}^3$ such that $s_x \leq x \leq s_x + x_C$, $s_y \leq y \leq s_y + y_C$ and $s_z \leq z \leq s_z + z_C$. We denote the set of all cuboids by O_0.*

We are interested in 3D structures that are more complicated than order-0 cuboids, in particular 3D structures that can have *tunnels*, that is, "holes".

▶ **Definition 9** (Order-1 cuboid)**.** *An* order-1 cuboid *C_1 is the difference between two elements of O_0. Given $C_0 = (s_{C_0}, x_{C_0}, y_{C_0}, z_{C_0})$ and $C'_0 = (s_{C'_0}, x_{C'_0}, y_{C'_0}, z_{C'_0})$ in O_0. $C_1 = C_0 \setminus C'_0$ is an order-1 cuboid if there is a $i \in \{x, y, z\}$ such that $i_{C_0} \leq i_{C'_0}$. We note O_1 the set of all order-1 cuboids.*

The genus of an order-1 cuboid is at most 1. The set of order-0 cuboids is a subset of the set of order-1 cuboids, that is, $O_0 \subseteq O_1$. An order-1 cuboid $C_1 = C_0 \setminus C'_0$ can be of three different types, depending on how C_0 and C'_0 interact: (i) C_0 and C'_0 have no intersection, and C_1 is an order-0 cuboid, (ii) C'_0 cuts a hole in C_0, and C_1 is denoted as an order-1 cuboid with a *tunnel* and has genus 1; and (iii) C_0 and C'_0 intersect but C'_0 does not cut a hole in C_0. If the cut is in the inner face of C_0, C_1 is an order-1 cuboid with a *pit* and if the cut is in the side of a face of C_0, C_1 is an order-1 cuboid with a *concavity*. In both cases of order-1 cuboid with a pit or with a concavity, the genus is 0.

3 Finding the Middle of a Rectangle

Our main results uses some arithmetic and geometric computations, which are defined in $\mathbb{Z}^2$. A transfer lemma guarantees that they also work on any surface, if it is regular enough.

Given an assembly of SFTAM on $\mathbb{Z}^2$, the smallest axis-parallel rectangle containing the assembly is its *underlying rectangle*. If an SFTAM assembly is on a 3D surface, it is permitted to fold along the tiles' edges. The underlying rectangle is then the smallest subset of the surface which contains the assembly and is isomorphic to a rectangle of $\mathbb{Z}^2$, if it exists.

▶ **Lemma 10.** *Let α be a producible assembly of an SFTAM TAS S on $\mathbb{Z}^2$ with underlying rectangle R, and let C be a polycube. If there exists a function $i : \mathbb{Z}^2 \to C$ such that the restriction of i to R is a graph isomorphism, then the image of α under i is producible on C.*

Proof. If the seed is placed at p_s in $\mathbb{Z}^2$, it is placed at $i(p_s)$ on C. Since the tile bonds can fold along edges of C, the assembly on C proceeds exactly as in $\mathbb{Z}^2$, and each tile placed at a point p in $\mathbb{Z}^2$ is placed at point $i(p)$ on C. ◀

We design the following systems (details omitted due to space constraints) which are a variant of those of [11]. The IBC (Increasing Binary Counter) counts up to a number, while distinguishing rows which are a power of two. The DBC (Decreasing Binary Counter) counts down from a number, while distinguishing the row where 0 is reached. The U-Turn System makes a copy of a number from position $[(x, y), ..., (x + k, y)]$ to position $[(x - k - 1, y), ..., (x - 1, y)]$. See Figure 15 for an example of IBC and DBC Systems, and Figure 16 for U-Turn System.

An *explicitly bounded rectangle R* is a rectangle whose horizontal sides are bounded by specially marked tiles. The following lemma uses the IBC, DBC and U-Turn Systems in a SFTAM TAS that finds the middle of the height of R.

▶ **Lemma 11** (Middle finding system)**.** *Let R be an explicitly bounded rectangle of height N and width at least $3\log(N)$. There is a TAS $S_{1/2} = (\Sigma, T_{1/2}, \sigma_+, str, \tau)$ such that for all assemblies with a seed located at the start, a tile of type t_m appears at coordinate $(x, \lfloor \frac{N}{2} \rfloor)$ with no other tiles to its left, and t_m does not appear anywhere else.*

Proof sketch. Let R be an explicitly bounded rectangle and $N = 2^n + k$ with $k < 2^n$ be the height of R. Without loss of generality, we assume that the specially marked horizontal sides of R being "Start" at the bottom and the other, "Finish", at the top. See Fig. 3 for an overview.

We use the IBC, DBC and U-Turn Systems to define our Middle Finding SFTAM TAS that finds $\frac{N}{2} = 2^{(n-1)} + \frac{k}{2}$. There are two copies of the IBC, named $IBC1$ and $IBC2$. We list the steps for the Middle Finding System, and omit the details due to space constraints.
0. Growing a column of support tiles until "Finish".

1. Using the IBC System $IBC1$ to find the height $N = 2^n + k$ of R. The support tiles from the previous step are its seed.
2. Returning to row number 2^n using the second IBC system $IBC2$, which then outputs the value of k
3. Copying k until 2^{n-1} (the middle of 2^n).
4. Halving k by eliminating its least significant bit.
5. Shifting $\frac{k}{2}$ to the left by a U-Turn System.
6. Going up by $\frac{k}{2}$ using the DBC system. ◀

Figure 3 The steps of the Middle Finding System process, starting from the seed that is in red on the right. The tile t_m (the red tile on the left) appears in the middle of two rows of tiles that are shown by start and finish.

4 Distinguishing order-1 cuboids by their genus

Our main result is stated as follows.

▶ **Theorem 12** (Main Theorem). *There is a SFTAM tile self-assembly system* $\mathcal{S}_\mathcal{G} = (\Sigma, T, \sigma, str, \tau)$ *and a subset of tile-types* $Y = \{t_{reg}, t_{mfs}\} \cup T_{ibc} \subseteq T$ *such that for any order-1 cuboid* $C = C_0 \setminus C_0'$ *with the dimensions at least 10 for* C_0'*, if* $\mathcal{S}_\mathcal{G}$ *assembles on* C *starting from a seed which is placed in a normal placement, the following holds:*

- *If* C *has genus* 1*, every terminal assembly of* $\mathcal{S}$ *on* C *contains at least one tile of* Y*.*
- *If* C *has genus* 0*, then no tile of* Y *appears in any producible assembly of* $\mathcal{S}$ *on* C*.*

The system presented here works for the case where the seed is on a *normal placement*, i.e. "far enough" from the borders of the surface.

▶ **Definition 13** (Normal placement). *Let* C *be an order-1 cuboid such that* (x_1, y_1, z_1), (x_2, y_2, z_2), ... ,(x_n, y_n, z_n) *are its vertices. A placement* $p \in Pl(C)$ *with position* (x, y, z) *is a* normal placement *of* C *if and only if for all* $i \in \mathbb{N}$*, two of the following inequalities hold:*

$\mid x_i - x \mid \geq 3\log(N)+6$, $\mid y_i - y \mid \geq 3\log(N)+6$ and $\mid z_i - z \mid \geq 3\log(N)+6$, where N is the largest of the three dimensions of the cuboid. The set of all normal placements is denoted by $Pl_N(C)$.

The simplest example to demonstrate the concept of normal placement is on an order-0 cuboid $C \in O_0$. In this case, normal placements consist of the cuboid's surface minus its "frame" i.e. the border of the cuboid's edges with a thick margin. Hence there are 6 disconnected areas on C's faces where the normal placements are. The normal placements on order-1 cuboids can be described similarly. It should be noted that in this case there can be more than 6 disconnected areas. Note that in order to have normal placements at all, a cuboid needs to be large enough. Also, when dimensions of the order-1 cuboid are large enough and not too disproportionate, most placements are normal placements.

4.1 Region partition on order-1 cuboids

Let $C = C_0 \setminus C_0'$ be an order-1 cuboid. In order to detect a potential tunnel whose entrances are on parallel faces, the construction separates these faces. For this purpose we use three planes, one for each pair of parallel faces of C, located between them. Let P_X, P_Y, P_Z be three planes in this way: take $p \in Pl_N(C)$. The plane P_X is passing on p and is parallel to the plane formed by the Y-axis and Z-axis. The plane P_Y is parallel to the plane formed by the X-axis and Z-axis and is passing on p. The plane P_Z is parallel to the plane formed by the X-axis and Y-axis and contains the center of C_0. In Fig. 4 the seed in yellow is in the point p and the plane P_X, P_Y and P_Z are framed respectively by the ribbons R_X (in red), R_Y (in green) and R_Z (in blue) on C. For $i \in \{X, Y\}$, R_i is the connected component of $\partial C \cap P_i$ that contains p. If R_X and R_Y intersect in one point, R_Z in the empty set. If they intersect in two points, $R'_Z = P_Z \cap \partial C$ and R_Z is the connected component of R'_Z that has an intersection with R_Y. The difference $C \setminus \{R_X, R_Y, R_Z\}$ consists of up to 8 connected components called *regions*. They are noted by R_{XYZ} such that $X, Y, Z \in \{0, 1\}$ where 0 represents the left, down and back sides, and 1 represents the right, up and front sides. For example, R_{101} refers to the region at the right, down and front side of C. The parity of the regions R_{XYZ} is the parity of $X+Y+Z$. This way of partitioning C helps to define the graph G_C, the *region graph* of C:

▶ **Definition 14** (Region graph). *Let $C = C_0 \setminus C_0^{'}$ be an order-1 cuboid with p as a position of it, the planes P_X, P_Y two perpendicular planes passing on p, and P_Z a plane perpendicular to both planes passing through the middle of P_Y. Also, let $R_i = \partial C_0 \cap P_i$ for $i \in \{X, Y, Z\}$. There is a graph assigned to C named the* region graph *$G_C(p)$ whose vertices are the regions separated by R_X, R_Y and an edge is added between two regions if and only if they share P_X, P_Y or P_Z.*

For an order-0 cuboid C, $G_C(p)$ is a bipartite graph and therefore it is 2-colorable. The region graph for an order-0 cuboid is presented in Fig. 7.

If C is an order-1 cuboid with a tunnel, the number of disconnected regions can be less than 8 depending on the intersection of the tunnel with the three planes P_X, P_Y and P_Z. The *axis* of the tunnel is the direction orthogonal to its entrances. The three planes can intersect the tunnel in two ways: along the width of the tunnel when the plane is perpendicular to the axis of the tunnel, or along the length of the tunnel when the plane is parallel to the axis of the tunnel. Thus, a tunnel may have an intersection with up to three perpendicular planes, one along the width, and up to two other planes along the length. Based on this, three types of partitions into regions are possible and the possible numbers of regions are: 7

regions when one plane intersects along the width of the tunnel, 5 regions when one plane intersects along the length of the tunnel and one along the width (See Fig 11), and 1 region when the three perpendicular planes intersect along the tunnel, one along the width and the others along the length.

4.2 Overview of the assemblies of the genus detector $\mathcal{S}_{\mathcal{G}}$ on O_1

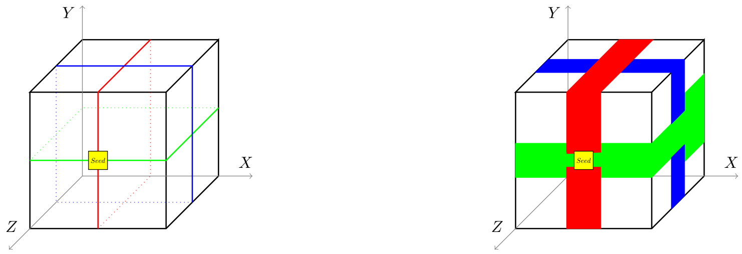


Figure 4 The skeleton of a terminal assembly of $\mathcal{S}_{\mathcal{G}}$ on an order-0 cuboid starting from a seed (in yellow) in a normal placement. On the left, the traces of the ribbons R_X (in red), R_Y (in green) and R_Z (in blue). On the right, the shape of the skeleton on the cuboid.

Let C be an order-1 cuboid. An assembly of $\mathcal{S}_{\mathcal{G}}$ starts from a seed in an arbitrary normal placement on C. In the TAS $\mathcal{S}_{\mathcal{G}}$, the seed acts like a compass for the assemblies. Without loss of generality, we assume that the side on which the seed is located is the face parallel to the XY-plane and intersects the Z axis, and the north label of the seed's tile points towards the Y axis. The process of the assemblies' growth in S_G consists of two phases, a phase for forming a *skeleton*, and a phase for filling up the skeleton:

1. Constructing the skeleton of the assembly's structures by at most 7 perpendicular ribbons on C. Here, the planes P_X, P_Y and P_Z are located from being framed by several ribbons of tiles (R_X, R_Y and R_Z) during the assembly and each step starts only when the previous step is finished.
   - R_X including one ribbon for framing the first plane P_X
   - R_Y including two ribbons for framing the second plane P_Y
   - R_Z including zero or four ribbons constitutes the frame of the third plane P_Z (depending on the intersection of the two previous planes, details will be given later)
2. Filling the inside of the assembly's skeleton by distinctive tiles. In this step the interior of the regions is partially filled by their distinctive tiles in a way that no connected component has a neighbor with the same inner filling tile.

In order to simplify the explanation of the process of the assemblies, first phase one is presented:
- how the skeleton grows depends on the placement of the seed
- how the skeleton partitions C into distinct connected components
- what its assigned region graph is.

Next, we study the phase of inner filling. Afterwards, we conclude the proof of the main theorem.

4.2.1 Terminal assemblies on order-0 cuboids

This section will characterise the set $A_{\square}^{C_0}[\mathcal{S}_{\mathcal{G}}]$ of terminal assemblies on an order-0 cuboid C_0. In fact, for the study of the shape of the productions in O_1^t, the productions on O_0 will be useful as a reference. We show that $\mathcal{S}_{\mathcal{G}}$ partitions order-0 cuboids into eight distinct regions as presented in Section 4.1. Later in the next section we study the case of order-1 cuboids.

1. The structure of the skeleton.

▶ **Lemma 15.** *Let $C \in O_0$ be an order-0 cuboid and assume that the seed σ is placed at a normal placement $p \in Pl_N(C)$. Every terminal assembly of $\mathcal{S}_\mathcal{G}$ on C includes a "3-step skeleton" noted by $R_X \cup R_Y \cup R_Z$ where each part is located on the corresponding ribbon defined in Section 4.1.*

Proof. Every terminal assembly of $\mathcal{S}_\mathcal{G}$ on C includes a "3-step skeleton" denoted by $R_X \cup R_Y \cup R_Z$ where each part is located on the corresponding ribbon defined in Section 4.1. In the first step, tiles make a vertical segment ribbon of tiles around C to form R_X, starting from the south of the seed and finishing at its north. R_X divides C into two regions, the *right side* and the *left side* of C with respect to the seed σ. (We always assume that we view the cuboid from the point of view of the Z-axis, as in Fig. 4, and thus *left*, *right*, *up*, *down*, *back*, *front* refer to this point of view.) Next, R_Y starts to form only when R_X rebounds. R_Y consist of two segment ribbons starting from both the right and left sides of σ. They develop perpendicular to R_X by using two Middle Finding Systems (Definition 11), one on each side. Note that each system has its own distinguished tile types. Once the R_Y ribbons form, they separate C into an *up side* and a *down side*. Thus, C is now partitioned into four separate regions due to the first and second step ribbons. Next, by finding the middle of each of R_Y's ribbons on the right and left faces, four new perpendicular ribbons are generated at the right-up and left-down sides from the tile of type t_m in the Middle Finding Systems. They go on, until they reach R_X on the upper and down faces. The plane R_Z passes through the union of these four ribbons. This step creates a separation between the *front side* and the *back side* of the cuboid C with respect to σ. We show the detailed assembly of R_X, R_Y and R_Z in Fig. 6 that starts from the seed σ (in red) at the left of the figure, where R_X rebounds on C. The western two-side Middle Finding System and its assign parts of R_Z is omitted for the sake of brevity, however they are the mirror image of the eastern ones. ◀

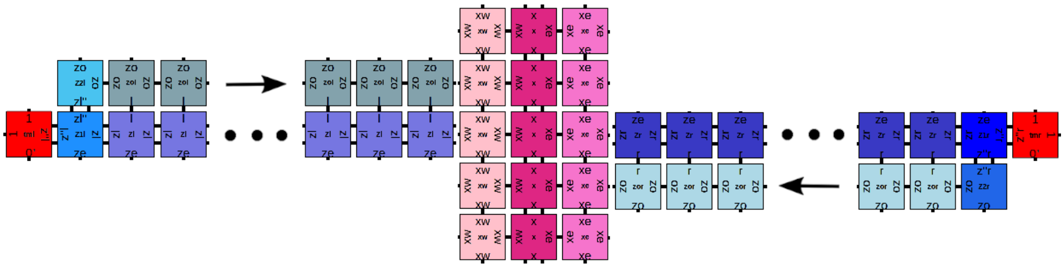


Figure 5 Two ribbons of R_Z meeting the ribbon R_X.

As mentioned in Section 4.1, these three steps partition C into 8 distinct regions.

2. Inner filling of the skeleton. After the formation of the skeleton, the second phase is to fill the eight regions by lines of interior tiles, once the R_Z ribbons reach R_X. Since the region graph of an order-0 cuboid C is a 2-colorable graph, we use two tile types to distinctly tile C.

▶ **Lemma 16.** *Let $C \in O_0$ be an order-0 cuboid. For all the terminal assemblies $\alpha \in A_\square^{C_0}[\mathcal{S}_\mathcal{G}]$ started from a seed $\sigma \in Pl_N(C)$, the tile $t_{even} = (z_e, x_{ev}, z_e, x_{ev})$ appears in the even regions and the tile $t_{odd} = (z_o, x_{od}, z_o, x_{od})$ appears in the odd regions.*

Proof. First, four ribbons of tiles types (see Fig. 8) appear at the intersection of P_X and the P_Z ribbons. Then, from the ribbons along R_X, straight lines of tiles start growing parallel to the x axis using strength 2 glues x_{ev} (resp. x_{od}) in even (resp. odd) regions. Thanks to

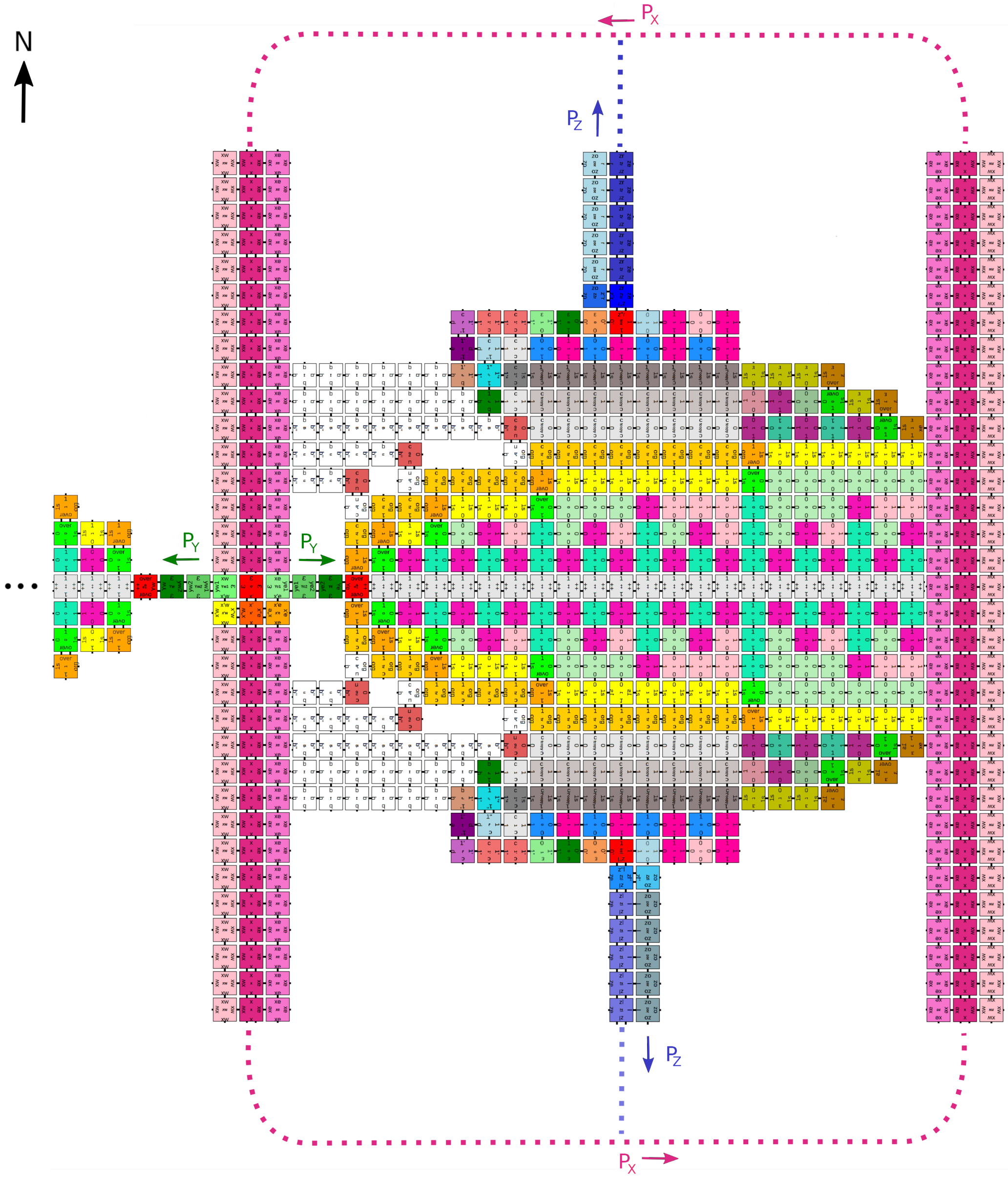


Figure 6 The assembly of R_X, R_Y and R_Z on an order-0 cuboid. The seed is located in the middle of R_X at the left. R_X grows from the south of the seed and finishes at its north. Then, P_Y starts growing by two two-side eastern and western Middle Finding Systems. At the end, P_Z starts to assemble from the found middle tile of R_Y (in red) and finishes by arriving at P_X. The western two-side Middle Finding System and its assigned parts of R_Z are omitted for the sake of brevity, however they are the mirror image of the eastern ones.

modulo 5 counters on the even R_X border tiles, there is one such line every other 5 position along that part of the border with tiles of type $t_{even} = (z_e, x_{ev}, z_e, x_{ev})$. Also, on the odd R_X border tiles, thanks to modulo 3 counters on the odd R_X border tiles, there is one such line every other 3 position along that part of the border with tiles of type $t_{odd} = (z_o, x_{od}, z_o, x_{od})$. These lines form the even (resp. odd) filling tiles and fill the partitioned regions. See Fig. 10 for an illustration.

(a) The region graph of an order-0 cuboid.

(b) Tile coloring of the region graph, and filling up the regions via tiles of types t_{even} and t_{odd}.

Figure 7 The region graph G_C and its 2-coloring.

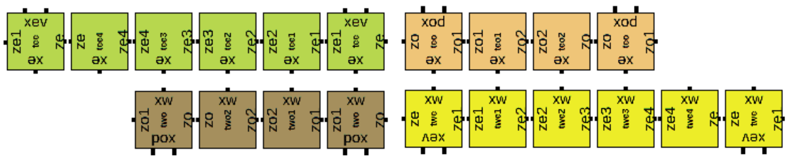


Figure 8 The tile types of four inner ribbons at the intersection of R_X and R_Z.

The 2-coloring indicates also where the tiles of type t_{even} and of type t_{odd} can be placed. The region R_{000} and the regions with even distance to it are tiled by tiles of type t_{even}, and the ones with odd distance to it, by t_{odd} tiles. For more clarity, see Fig. 7 where the regions corresponding to t_{even} are colored white and the ones with t_{odd} are colored black. ◀

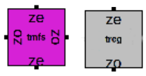


Figure 9 The tile types t_{mfs} and t_{reg} (which may only appear if C has genus 1).

4.2.2 Terminal assemblies on order-1 cuboids with genus 1

We consider now the process of the assembly of $\mathcal{S}_\mathcal{G}$ for order-1 cuboids with a tunnel. This section will characterise the set $A_\square^{C_1^t}[\mathcal{S}_\mathcal{G}]$ of terminal assemblies on such order-1 cuboids.

Let C be an order-1 cuboid with a tunnel. The key element of the proof is the appearance of some specific tile in each assembly since it has less than 8 regions. The assemblies on C have a skeleton with a different shape depending on the region graph associated with the placement of the seed. Let P_i and R_i for $i \in \{X, Y, Z\}$ be defined as presented in Section 4.1. If a plane P_i intersects along the width of the tunnel, it acts like a separator between the two parallel faces where the tunnel's entrances are located. If a plane P_i intersects along the length of the tunnel, the tiles of R_i enter and pass inside the tunnel. Moreover, three types of partitions into regions are possible and the possible numbers of regions are: 7 regions when one plane intersects along the width of the tunnel, 5 regions when one plane intersects along the length of the tunnel and one along the width, and 1 region when three perpendicular planes intersect along the tunnel, one along the width and the others along the length. Each case needs to be studied separately, we give the proof of the case with 5 regions and the proof of the Lemma 17 and Lemma 19 exist in the appendix.

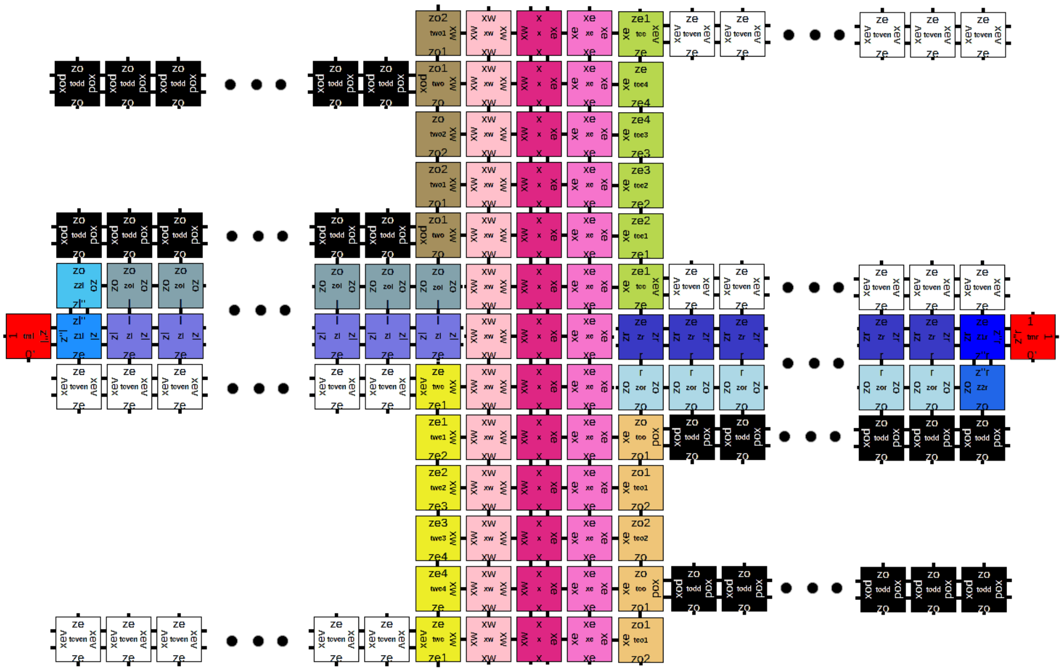


Figure 10 The inner filling with tiles of types t_{even} (white) and t_{odd} (black) at the two places where the R_Z ribbons meet R_X.

Note that in what follows, $G_C(\sigma)$ refers to the region graph $G_C(p)$ such that p is the position of the seed σ on C.

Case 1 (7 regions): one plane intersects along the width of the tunnel. In this case, tiles of types t_{odd} and t_{even} touch, which enforces the attachment of t_{reg} or t_{mfs}.

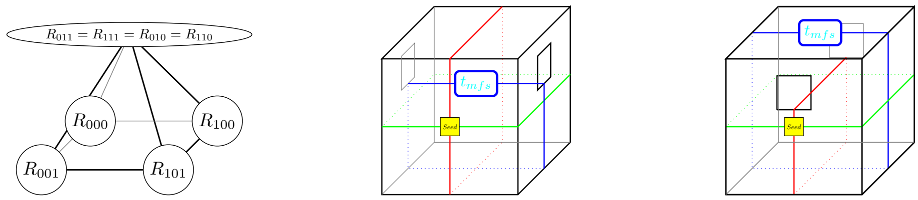
▶ **Lemma 17.** *Let $C = C_0 \setminus C'_0 \in O_1^t$ be an order-1 cuboid with the dimensions at least 10 for C'_0. Assume that the seed σ is placed in a normal placement $p \in Pl(C)$. In a terminal assembly of the system $S_\mathcal{G}$, if only one of the planes defined in Section 4.1 intersect with the tunnel, $G_C(\sigma)$ has 7 regions and a tile of type t_{reg} or t_{mfs} appears in the assembly.*

Case 2 (5 regions): the tunnel intersects with P_Z, and exactly one of P_X and P_Y.

▶ **Lemma 18.** *Let $C \in O_1^t$ be an order-1 cuboid and assume that the seed σ is placed in a normal placement $p \in Pl(C)$. In a terminal assembly of the system $S_\mathcal{G}$, if the plane P_Z and exactly one of the planes P_X and P_Y defined in Section 4.1 have an intersection with the tunnel, there exist 5 regions on the cuboid and a tile of type t_{mfs} appears in the assembly.*

Proof. If the seed is placed where the tunnel has intersection with two perpendicular planes, one of them intersects the tunnel along its width and the other one along its length. If P_Z intersects with the tunnel along the length, the ribbons of R_Z meet each other inside the tunnel. However, if P_Z intersects the tunnel along its width, they meet outside the tunnel.

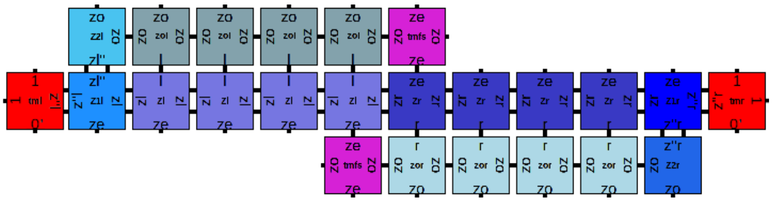
In both cases, the tile $t_{mfs} = (z_e, z_o, z_e, z_o)$ appears in the assembly when two frame ribbons of P_Z meet each other. Note that when the tunnel has intersection with P_Z and one of the planes P_X or P_Y, the cuboid is separated into two connected components such that one of them is a cuboid with genus 0 and the other one is a cuboid with genus 1. The part with genus 0 has 4 distinct regions, and the part with genus 1 (containing a tile of type t_{mfs}) has one single region. In total, there exist 5 distinct regions on the cuboid C. For an illustration of the skeleton and its graph in this case, see Fig. 11. ◀



(a) The region graph.

(b) The tunnel intersects along the width of plane P_X and length of plane P_Z.

(c) The tunnel intersects along the length of plane P_X and width of plane P_Z.



(d) A tile of type t_{mfs} appears if and only if two segments of P_Z (in purple) intersect each other by passing through a tunnel (instead of reaching P_X).

Figure 11 The case where C is partitioned into 5 distinct regions.

Case 3 (1 region): the tunnel intersects with P_X and P_Y. Here, two opposite ribbons of R_Y meet instead of colliding with R_X. This enforces the attachment of some tiles in $T_{ibc} \subseteq Y$.

▶ **Lemma 19.** *Let $C \in O_1^t$ be an order-1 cuboid and assume that the seed σ is placed in a normal placement $p \in Pl(C)$. In a terminal assembly of the system $\mathcal{S}_\mathcal{G}$, if two planes P_X and P_Y defined in Section 4.1 intersect the tunnel, there exists 1 regions on the cuboid and a tile of one of the T_{ibc} types appears in the assembly.*

Note that the situation when the seed is located inside the tunnel is similar to Case 3, up to topological isomorphism.

From Lemmas 17, 18 and 19, the following corollary is obtained:

▶ **Corollary 20.** *Let $C = C_0 \setminus C_0' \in O_1$ be an order-1 cuboid with the dimensions at least 10 for C_0' and α be an assembly of the TAS $\mathcal{S}_\mathcal{G} = (\Sigma, T, \sigma, str, \tau)$ such that its seed is placed at a normal placement. If there is tunnel on C (i.e. its genus is 1), at least a tile type from $Y = \{t_{reg}\} \cup \{t_{mfs}\} \cup T_{ibc} \subseteq T$ exists in all terminal assemblies of $\mathcal{S}_\mathcal{G}$ on C.*

4.3 Detecting the genus of the order-1 cuboids via $\mathcal{S}_\mathcal{G}$

Before proving our main theorem (Theorem 12), we need to prove the following lemma:

▶ **Lemma 21.** *Let C be an order-1 cuboid. If one tile of Y=$\{t_{reg}\} \cup \{t_{mfs}\} \cup T_{ibc} \subseteq T$ exists in a terminal assembly of $\mathcal{S}_\mathcal{G}$ on C starting from a seed in a normal placement, there is a tunnel on C.*

Proof. If a tile of type t_{mfs} exists in a terminal assembly on C, two cases are possible. In one case, there is a tunnel that intersects only P_X along its width and t_{even} and t_{odd} intersect each other perpendicularly. As a result, t_{mfs} appears in the assembly. In the other case, two ribbons of R_Z must meet each other since the tiles whose labels correspond to the labels of t_{mfs} are those of the R_Z ribbons. Recall that the R_X and R_Y ribbons intersect at two places : one at the seed (since R_Y grows out of R_X) and a second time, where the tiles of type t_{eu}, t_{ed}, t_{wu} or t_{wd} appear in the assembly as the row tile number 1, in the second IBC system of the Middle Finding Systems. The two ribbons of R_Z, together with the parts of the Middle Finding System located between the second intersection of R_X and R_Y on the one hand, and R_Z on the other hand, form a closed ribbon on the surface of C (highlighted in green and blue Fig. 12). This ribbon and R_X pass through each other perpendicularly at only one place. Since they pass through each other perpendicularly, it can be concluded that the cuboid C cannot be topologically homeomorphic to the sphere, or in other words, be a genus 0 cuboid, and so a tunnel must exist. The cases where there is a tile of type t_{reg} or $t \in T_{ibc}$ are similar. ◀

In the cases where the genus is 0 but there is a pit or concavity the construction also yields an 8 region partition but the details are omitted due to space constraints.

We are now ready to prove Theorem 12.

Proof of Theorem 12. Let $C = C_0 \setminus C'_0 \in O_1$ be an order-1 cuboid with the dimensions at least 10 for C'_0 and α be an assembly of the TAS $\mathcal{S}_{\mathcal{G}} = (\Sigma, T, \sigma, str, \tau)$ such that its seed is placed at a normal placement. Note that if C_0 is too small, there is no normal placement. By Corollary 20 and Lemma 21, there is a tile type from $Y = \{t_{reg}\} \cup \{t_{mfs}\} \cup T_{ibc} \subseteq T$ in all terminal assemblies of $\mathcal{S}_{\mathcal{G}}$ on C if and only if there is tunnel on C (i.e. its genus is 1). ◀

Figure 12 The closed ribbon formed by parts of the Middle Finding System (green) and the two ribbons of R_Z (blue), when two R_Z ribbons meet each other instead of reaching R_X. They meet the red ribbon R_X only once.

The general principle of the construction is as follows: cut the order-1 cuboid into regions and check if the partition is the same as it would be on a cube. If it is the case, the cuboid has genus 0, eight regions and the tiles of Y cannot be used in any terminal assembly. Otherwise, the cuboid has genus 1, less than eight regions, and the tiles of Y must be used in any terminal assembly.

In fact, the SFTAM system we obtain works quite intuitively. The different "moving parts" are necessary to ensure the good function of the system:

- Firstly, the Middle Finding System ensures the shape is split along each dimension. In fact, a precise control is necessary to prevent false positives, i.e. order-1 cuboids of genus 0 with a tile of Y in their assembly, and less than 8 regions. To do so, the partition ensures that any tunnel lies between two different regions that have the possibility of sharing a tunnel. Hence, the Middle Finding System is needed to avoid false positives.
- Lastly, the filling with unequally spaced stripes ensures that there is enough empty space which triggers the detection of the meeting of two regions.

The relative complexity of these illustrates the challenges of working on an unknown surface.

5 Conclusion

We have introduced our new model, SFTAM, that enables to perform tile self-assembly on 3D surfaces. We have shown that we can use it to determine the genus of a given surface. For this, we have worked on a simple and special family of polycubes, the order-1 cuboids.

It would be interesting to extend our results to a larger family of polycubes. In our work, the Middle Finding System was used to detect a potential tunnel on an order-1 cuboid. However, for more complicated surfaces, one needs to ensure that some part of the construction does go through the tunnel, and that it can be differentiated from the tiles it meets on the other side. The idea of having regions with distinct identities can be reused in this context, but the Middle Finding System needs to be supplemented or replaced.

References

1 Zachary Abel, Nadia Benbernou, Mirela Damian, Erik D Demaine, Martin L Demaine, Robin Flatland, Scott D Kominers, and Robert Schweller. Shape replication through self-assembly and rnase enzymes. In *Proceedings of the twenty-first annual ACM-SIAM symposium on discrete algorithms*, pages 1045–1064. SIAM, 2010.

2 Greg Aloupis, Prosenjit K Bose, Sébastien Collette, Erik D Demaine, Martin L Demaine, Karim Douieb, Vida Dujmović, John Iacono, Stefan Langerman, and Pat Morin. Common unfoldings of polyominoes and polycubes. In *Computational Geometry, Graphs and Applications: 9th International Conference, CGGA 2010, Dalian, China, November 3-6, 2010, Revised Selected Papers*, pages 44–54. Springer, 2011.

3 Robert D Barish, Rebecca Schulman, Paul WK Rothemund, and Erik Winfree. An information-bearing seed for nucleating algorithmic self-assembly. *Proceedings of the National Academy of Sciences*, 106(15):6054–6059, 2009.

4 Matthew Cook, Tristan Stérin, and Damien Woods. Small Tile Sets That Compute While Solving Mazes. In *27th International Conference on DNA Computing and Molecular Programming (DNA 27)*, volume 205 of *Leibniz International Proceedings in Informatics (LIPIcs)*, pages 8:1–8:20. Schloss Dagstuhl – Leibniz-Zentrum für Informatik, 2021. doi:10.4230/LIPIcs.DNA.27.8.

5 Jérôme Durand-Lose, Jacob Hendricks, Matthew J Patitz, Ian Perkins, and Michael Sharp. Self-assembly of 3-d structures using 2-d folding tiles. *Natural Computing*, 19:337–355, 2020.

6 Matthew R Jones, Nadrian C Seeman, and Chad A Mirkin. Programmable materials and the nature of the dna bond. *Science*, 347(6224):1260901, 2015.

7 Wenyan Liu, Hong Zhong, Risheng Wang, and Nadrian C Seeman. Crystalline two-dimensional dna-origami arrays. *Angewandte Chemie International Edition*, 50(1):264–267, 2011.

8 Kao Ming-Yang and Vijay Ramachandran. Dna self-assembly for constructing 3d boxes. In *International Symposium on Algorithms and Computation*, pages 429–441. Springer, 2001.

9 Matthew J Patitz. An introduction to tile-based self-assembly and a survey of recent results. *Natural Computing*, 13:195–224, 2014.

10 Paul WK Rothemund. Folding dna to create nanoscale shapes and patterns. *Nature*, 440(7082):297–302, 2006.

11 Paul WK Rothemund and Erik Winfree. The program-size complexity of self-assembled squares. In *Proceedings of the thirty-second annual ACM symposium on Theory of computing*, pages 459–468, 2000.

12 Ned et al." "Seeman. Dna nanotechnology: Bibliography from ned seeman's laboratory, 2023. URL: http://seemanlab4.chem.nyu.edu/nanobib.html.

13 Vyankat A Sontakke and Yohei Yokobayashi. Programmable macroscopic self-assembly of dna-decorated hydrogels. *Journal of the American Chemical Society*, 144(5):2149–2155, 2022.

14 Hao Wang. Proving theorems by pattern recognition—ii. *Bell system technical journal*, 40(1):1–41, 1961.

15 Erik Winfree. *Algorithmic self-assembly of DNA*. California Institute of Technology, 1998.

16 Joseph F Woods, Lucía Gallego, Pauline Pfister, Mounir Maaloum, Andreas Vargas Jentzsch, and Michel Rickhaus. Shape-assisted self-assembly. *Nature Communications*, 13(1):3681, 2022.
17 Shuguang Zhang. Fabrication of novel biomaterials through molecular self-assembly. *Nature biotechnology*, 21(10):1171–1178, 2003.
18 Rebecca Zhuo, Feng Zhou, Xiaojin He, Ruojie Sha, Nadrian C Seeman, and Paul M Chaikin. Litters of self-replicating origami cross-tiles. *Proceedings of the National Academy of Sciences*, 116(6):1952–1957, 2019.

A Technical Details

In our assemblies, we will represent a number by tiles, in a classic way for tile self-assembly (see e.g. [11]) as follows.

▶ **Definition 22** (Row tile number). *Let T_0 and T_1 be two sets of tiles with labels 0 and 1, respectively. Let N be an integer and $a_1...a_2a_n$ be its binary representation with $n = \lceil log_2 N \rceil$. A row of tiles with labels $a_1^*, a_2, ..., a_n$ is the* row tile number *representation of N such that the distinct tile a_1^*, represents the most significant bit of the number. See Fig. 13 for an example.*

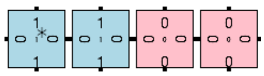
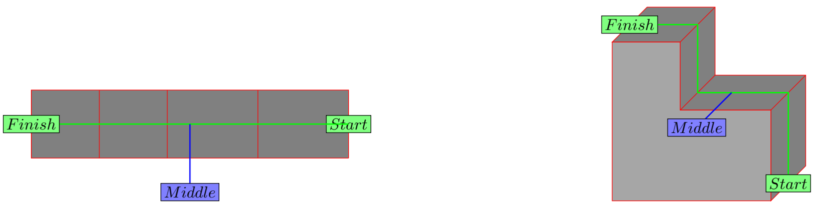


Figure 13 Representation of the number 12 by its row tile number.



(a) On $\mathbb{Z}^2$.   **(b)** On a polycube.

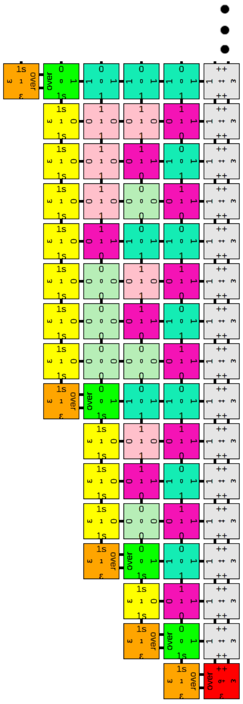
Figure 14 Finding the middle of a surface. The underlying rectangle is in dark gray.

B The order-1 cuboids with pit or concavity

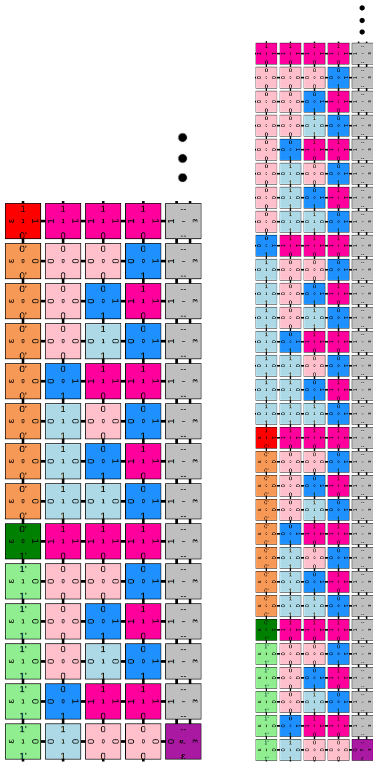
In the case that $C \in O_1$ is an order-1 cuboid with a concavity or a pit (whose genus is 0), the assembly's process is similar to the assembly on order-0 cuboids. The frame ribbons form completely by the assumption that the seed is located on a normal placement of C, and separate C into 8 distinct regions, and the insides of the regions are tiled independently by inner tiles of types t_{odd} and t_{even}. However, in the case of a concavity, the regions do not necessarily meet edge to edge, see Fig. 17 for an illustration.

C Omitted proofs

Proof of Lemma 17. Let the seed be placed in a manner that only one of the planes P_X, P_Y or P_Z intersects along the width of the tunnel.



(a) The assembly of the IBC System until the number 16.



(b) The assembly of the DBC System for number 12 to −1 at the left and negative 15 at the right.

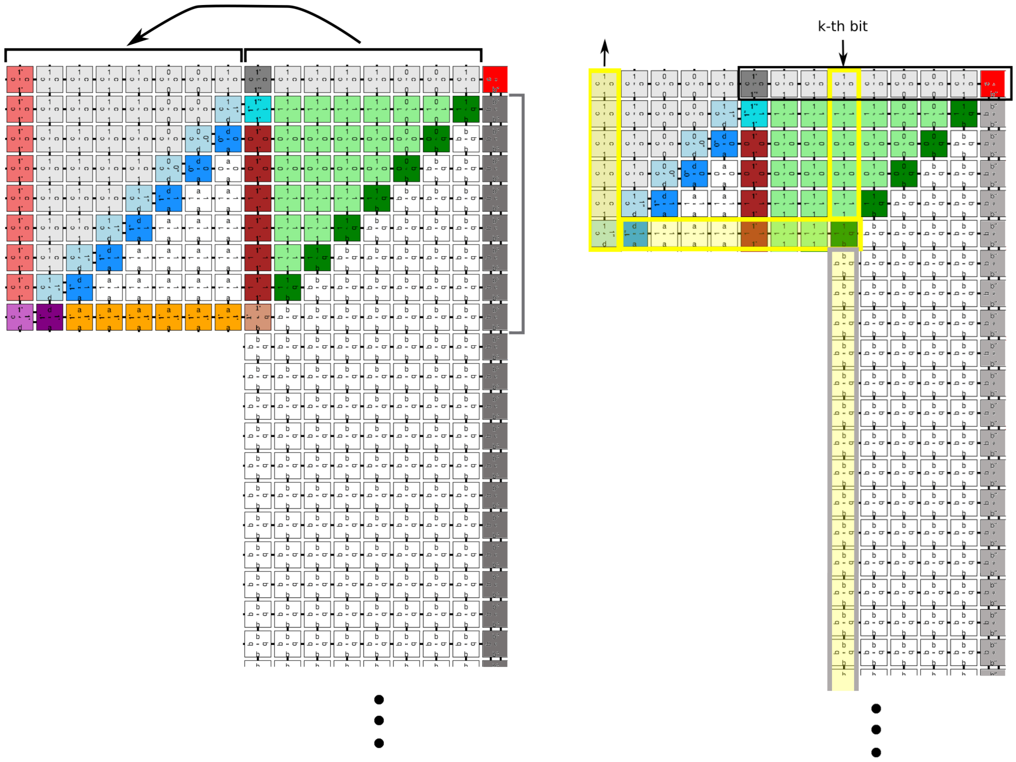
Figure 15 Increasing Binary Counter System (left) and Decreasing Binary Counter System (right).

The plane that intersects the tunnel is the separating buffer of two regions R_{xyz} and $R_{x'y'z'}$ containing the two tunnel's entrances. In this case, the two regions R_{xyz} and $R_{x'y'z'}$ are joined by the tunnel and get combined into a single region via the tunnel. Therefore, the number of distinct regions decreases to 7 regions (compared with the genus 0 case, where we always have 8 regions). See Fig. 18 for an illustration.

Without loss of generality, assume that $x + y + z$ is an odd number and $x' + y' + z'$ is an even number. When two regions R_{xyz} and $R_{x'y'z'}$ are joined by the tunnel, tiles of type $t_{odd} = (z_o, x_{od}, z_o, x_{od})$ from R_{xyz} and of type $t_{even} = (z_e, x_{ev}, z_e, x_{ev})$ from $R_{x'y'z'}$ both exist in the new unique region. We show that the tile type $t_{reg} = (z_e, x_{od}, z_o, x_{ev})$ or t_{mfs} must then occur in the assembly. The tile types t_{reg} and t_{mfs} are the only tile type of $\mathcal{S}_\mathcal{G}$ with labels z_o and z_e of inner filling tiles t_{odd} and t_{even}. To conclude the proof, one needs to show that in a region with a disconnected border, there is a *good* empty space, that is an empty space which sees both an even tile and an odd tile through strength 1 sides. Then, this space can be filled by neither type of filling tiles, but it must eventually be filled by a tile of type $t_{reg} = (z_e, x_{od}, z_o, x_{ev})$ or $t_{mfs} = (z_e, z_o, z_e, z_o)$. In a region with a tunnel, on each side of the tunnel, the border of every 10×10 square must be crossed by either

- at least two of the lines of tiles starting from P_X on that side of the tunnel, or
- at least two of the lines exiting the tunnel.

In particular, because C'_0 is at least 10×10 units wide, there are at least two lines crossing one of the edges the tunnel in the same direction. Each such line must either reach the



(a) Copying 11111001 in the U-Turn System. The gray bracket on the right shows the minimum number of support tiles that are necessary for this assembly.

(b) The k-th stage of the assembly in the U-Turn System is shown by yellow-filled rectangles. The value of the k-th significant bit is copied down by $k-1$ rows during the previous stages. The k-th stage copies the value one time to the south and n times to the left and finally k times to the top. Here, $k = 5$ and $n = 8$. In addition, in the k-th stage, the tiles of type $t^b_{\longleftarrow}$ in the gray rectangle appear below the tile of type $t_{\lrcorner}$, and they will be the supports for the $(k+1)$-th stage. The seed is highlighted in the black rectange.

Figure 16 U-Turn System.

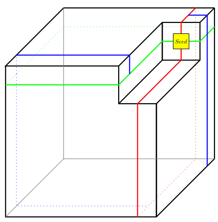


Figure 17 Cuboid with concavity: R_X (red), R_Y (green) and R_Z (blue). The latter consists of two semi-planes.

opposite connected component of the border, be stopped orthogonally by a line from the opposite side of the tunnel, or run head-first into an opposite line. Consider such a pair of lines, with minimal distance between them. In particular, that distance must be at most 10.

- If one of the lines reaches the opposite connected component of the border, either of the spaces next to its end is *good* and in this case the tile of type t_{reg} appears in the assembly;

- likewise, if one of them is stopped orthogonally by a line from the opposite side of the tunnel, one of the spaces next to the intersections is *good* and a tile of type t_{mfs} appears.

Moreover, if one of them runs head-first into an opposite line, the other cannot, because their distance cannot be at the same time divisible by 15, positive and less than 10. Hence the pair satisfies one the previous cases. This concludes the proof of that case of our construction. ◀

Proof of Lemma 19. In this case, the skeleton of the assembly is not the same as before. Recall the process of the assembly's skeleton: The frame ribbons of the plane P_X are generated independently from σ. Two segment ribbons of the plane P_Y begin to grow after rebounding on the plane P_X, regardless of passing through a tunnel or not. However, the ribbons of P_Z start to grow only after finding the middle of P_Y and they end by reaching the ribbon of P_X. Considering this process, when the two planes P_X and P_Y intersect with the tunnel, the plane P_Z is not able to form since there is a tunnel that does not permit to have the collision of P_Y and P_X, and the ribbons of P_Y do not end in P_X. As a result, since the plane P_Z depends on the collision of the P_Y ribbons with the P_X ribbons, P_Z is not able to form.

Moreover, two ribbons of P_Y must meet each other at a tile of one of the T_{ibc} types that comes between their $IBC1$ systems. This happens inside the tunnel if P_Y intersects the tunnel along its length, and outside the tunnel if it intersects the tunnel along its width. In either cases a tile of one of the T_{ibc} types appears.

Note that the skeleton consists of two closed loops of P_X ribbons and P_Y ribbons. This phenomenon demonstrates that the genus of C is 1. In order to have a better overview, see Fig. 19. Furthermore, all regions are united and there is only one single region throughout the whole surface of C. ◀

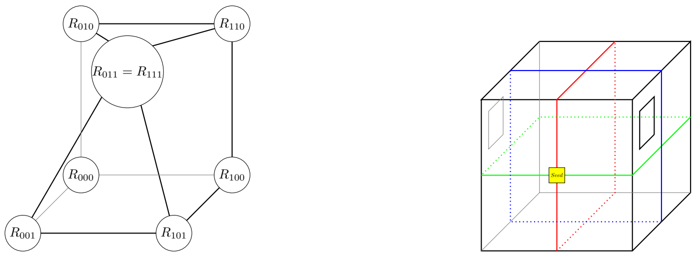
Proof of Corollary 20. If there is a tunnel on C, at least one of the planes P_X, P_Y and P_Z defined in Section 4.1 intersects with the tunnel since its entrances are on parallel faces of the cuboid, and these planes are located between parallel faces.

Firstly, if the tunnel of C intersects with only one of the planes, due to Lemma 17, a tile of type t_{reg} or t_{mfs}, which are the only tile types of $\mathcal{S}_{\mathcal{G}}$ with labels in common with both inner filling tile types t_{odd} and t_{even}, appears in the assembly.

Nextly, if two planes (among them P_Z) intersect with the tunnel on C, a tile of type t_{mfs} appears in all terminal assemblies on C by Lemma 18.

At the end, if two planes P_X and P_Y intersect with the tunnel, Lemma 19 implies that a tile of one of the T_{ibc} types is present in the assembly. ◀

The places where a tunnel implies the presence of a tile of Y are shown in Fig. 20.



(a) Region graph. **(b)** The cuboid and the three planes.

Figure 18 The case where $C \in O_1^t$ is partitioned into 7 distinct regions. If there is a tunnel between two distinct regions, a tile of type t_{reg} or t_{mfs}, which have common labels with both t_{even} and t_{odd}, must appear in the assembly.

(a) The tunnel intersects along the length of plane P_X and width of plane P_Y. **(b)** The tunnel intersects along the width of plane P_X and length of plane P_Y.

Figure 19 Intersection of tunnel with the two planes P_X (red) and P_Y (green).

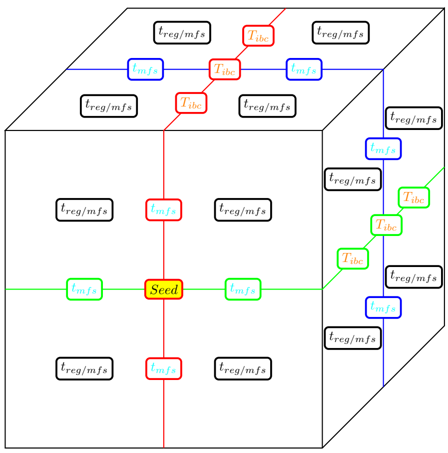


Figure 20 The places on a cuboid where, if there is a tunnel, a tile of Y must appear in the assembly.