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

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

Shahrzad Heydarshahi -envelope
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 subtracting 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.

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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-assembly 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 $Z^3$) 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 $Z^2$. 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
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) $S_0$ 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 $S_0$ on $C$, and
- if $C$ has genus 1, every terminal assembly of $S_0$ on $C$ contains at least one tile of $Y$.

In other words, the genus of $C$ can be determined using the assemblies of $S_0$ on $C$. The assemblies of $S_0$ 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.

Figure 1 The skeleton of a $S_0$ 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 $t_{\text{odd}}$ and $t_{\text{even}}$. 

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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 $\Sigma$ be a finite label alphabet and $\epsilon$ 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 cannot 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 $\Pi(C)$.

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 $\Sigma$. 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 $S = (\Sigma, T, \sigma, str, \tau)$, where:

- $\Sigma$ is a finite label alphabet,
T is a finite set of tile types on \( \Sigma \),
- \( \sigma \) is called the seed and can be a single tile or several tiles
- \( \text{str} \) is a function from \( \Sigma \cup \{\epsilon\} \) to non-negative integers called strength function such that \( \text{str}(\epsilon) = 0 \), 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 \( \text{str} \) show the strength of their connections and the \( \tau \) 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 \( \alpha \) of a SFTAM TAS \( S \) on a polycube \( C \) is a partial function \( \alpha: \text{Pl}(C) \rightarrow T \) defined on at least one placement such that for each facet \( f \) of \( C \), there is at most one placement \( (f, o) \) where \( \alpha \) is defined.

For placements \( p = (f, o) \), \( p' = (f', o') \) of \( \text{Pl}(C) \) 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 \( G_\alpha \) associated to \( \alpha \) has as its vertices, the placements of \( \text{Pl}(C) \) that have an image by \( \alpha \), and two placements \( p \) and \( p' \) are adjacent in \( G_\alpha \) if the tiles \( \alpha(p) \) and \( \alpha(p') \) bind.

An assembly \( \alpha \) is \( \tau \)-stable if for breaking \( G_\alpha \) to any smaller assemblies, the sum of the strengths of disconnected edges of \( G_\alpha \) needs to be at least \( \tau \).

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

The SFTAM assemblies start from a seed and growth by a tile 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 \( \tau \) 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 \( C_i \) 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 \( \alpha \) 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 \( \alpha \) 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 the row where 0 is reached. The U-Turn System makes a copy of a number from position \( (x,y) \) 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 in 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 \( S_{1/2} = (\Sigma, T_{1/2}, \sigma_+, \text{str}, \tau) \) 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.

1. Growing a column of support tiles until “Finish”.

...
1. Using the IBC System $IBC_1$ 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 $IBC_2$, 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.

\[ \geq \log(N) \]

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.

\[ \textbf{Theorem 12 (Main Theorem).} \] There is a SFTAM tile self-assembly system $S_G = (\Sigma, T, \sigma, \text{str}, \tau)$ 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 $S_G$ 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 $S$ 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 $S$ 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.

\[ \textbf{Definition 13 (Normal placement).} \] 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:
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 \( p \in P_N(C) \). 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, \( R_{001} \) 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 \( C \):

\[
\text{Definition 14 (Region graph). Let } C = C_0 \setminus C'_0 \text{ be an order-1 cuboid with } p \text{ as a position of it, the planes } P_X, P_Y \text{ two perpendicular planes passing on } p, \text{ and } P_Z \text{ a plane perpendicular to both planes passing through the middle of } P_Y. \text{ Also, let } R_i = \partial C_0 \cap P_i \text{ for } i \in \{ X,Y,Z \}. \text{ There is a graph assigned to } C \text{ named the region graph } G_C(p) \text{ whose vertices are the regions separated by } R_X, R_Y \text{ and an edge is added between two regions if and only if they share } P_X, P_Y \text{ or } P_Z.
\]

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 $S_G$ on $O_1$

Let $C$ be an order-1 cuboid. An assembly of $S_G$ starts from a seed in an arbitrary normal placement on $C$. In the TAS $S_G$, 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 $A^0_{O_0}[S_G]$ of terminal assemblies on an order-0 cuboid $C_0$. In fact, for the study of the shape of the productions in $O_1$, the productions on $O_0$ will be useful as a reference. We show that $S_G$ 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 $\sigma$ is placed at a normal placement $p \in PL_N(C)$. Every terminal assembly of $SG$ 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 $SG$ 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 $\sigma$. (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 $\sigma$. 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 $\sigma$. We show the detailed assembly of $R_X$, $R_Y$ and $R_Z$ in Fig. 6 that starts from the seed $\sigma$ (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 $\alpha \in A^T(SG)$ started from a seed $\sigma \in PL_N(C)$, the tile $t_{ev} = (x_e, x_{ev}, z_e, x_{ev})$ appears in the even regions and the tile $t_{od} = (x_o, x_{od}, z_o, x_{od})$ 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
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.
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(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 $t_{\text{even}}$ and $t_{\text{odd}}$.

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 $t_{\text{even}}$ and of type $t_{\text{odd}}$ can be placed. The region $R_{000}$ and the regions with even distance to it are tiled by tiles of type $t_{\text{even}}$, and the ones with odd distance to it, by $t_{\text{odd}}$ tiles. For more clarity, see Fig. 7 where the regions corresponding to $t_{\text{even}}$ are colored white and the ones with $t_{\text{odd}}$ 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 $S_G$ for order-1 cuboids with a tunnel. This section will characterise the set $A_{\text{C}}^1[S_G]$ 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 $t_{\text{even}}$ (white) and $t_{\text{odd}}$ (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 $\sigma$ on $C$.

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

*Lemma 17.* Let $C = C_0 \setminus C'_0 \in O_1$ be an order-1 cuboid with the dimensions at least 10 for $C'_0$. Assume that the seed $\sigma$ is placed in a normal placement $p \in Pl(C)$. In a terminal assembly of the system $S_G$, 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 $t_{\text{reg}}$ or $t_{\text{mfs}}$ 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 $C \in O_1$ be an order-1 cuboid and assume that the seed $\sigma$ is placed in a normal placement $p \in Pl(C)$. In a terminal assembly of the system $S_G$, 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 $t_{\text{mfs}}$ 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 $t_{\text{mfs}} = (z_e, z_o, z_e, z_o)$ 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 \(t_{mf}\)) 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.

\[ R_{000} = R_{101} = R_{001} = R_{100} = R_{010} \]

(a) The region graph.

(b) The tunnel intersects along the width of plane \(P_X\) and length of plane \(P_2\).

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

(d) A tile of type \(t_{mf}\) 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 \(T_{ibc} \subseteq Y\).

\[ \text{Case 3 (1 region): the tunnel intersects with } P_X \text{ and } P_Y \]  

\[ \text{Here, two opposite ribbons of } R_Y \text{ meet instead of colliding with } R_X. \text{ This enforces the attachment of some tiles in } T_{ibc} \subseteq Y. \]  

\[ \text{Note that the situation when the seed is located inside the tunnel is similar to Case 3, up to topological isomorphism.} \]

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

**Corollary 20.** Let \(C = C_0 \setminus C_0' \subseteq O_1\) be an order-1 cuboid with the dimensions at least 10 for \(C_0'\) and \(\alpha\) be an assembly of the TAS \(S_G = (\Sigma, T, \sigma, \text{str}, \tau)\) 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 \(Y = \{t_{reg}\} \cup \{t_{mf}\} \cup T_{ibc} \subseteq T\) exists in all terminal assemblies of \(S_G\) on \(C\).

### 4.3 Detecting the genus of the order-1 cuboids via \(S_G\)

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 \(Y = \{t_{reg}\} \cup \{t_{mf}\} \cup T_{ibc} \subseteq T\) exists in a terminal assembly of \(S_G\) 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. $\blacksquare$

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 $\alpha$ be an assembly of the TAS $S'_G = (\Sigma, T, \sigma, str, \tau)$ 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 $S'_G$ on $C$ if and only if there is tunnel on $C$ (i.e. its genus is 1). $\blacksquare$

**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


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 $a_1...a_n$ 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](image)

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

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.
The assembly of the IBC System until the number 16.

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 $t_{odd} = (z_0, x_{od}, z_0, x_{od})$ 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 $t_{reg} = (z_o, x_{od}, z_o, x_{ev})$ or $t_{mfs}$ must then occur in the assembly. The tile types $t_{reg}$ and $t_{mfs}$ are the only tile type of $SG$ 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 $t_{reg} = (z_e, x_{od}, z_0, x_{ev})$ or $t_{mfs} = (z_e, z_0, z_e, z_0)$. In a region with a tunnel, on each side of the tunnel, the border of every $10 \times 10$ 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 $10 \times 10$ 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 1111001 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 $t_{\uparrow}$ in the gray rectangle appear below the tile of type $t_{\leftarrow}$, and they will be the supports for the $(k + 1)$-th stage. The seed is highlighted in the black rectangle.

- **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 $t_{\text{reg}}$ 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 $\sigma$. 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 $IBC_1$ 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 $S_G$ 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.
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 $t_{\text{reg}}$ or $t_{\text{mfs}}$, which have common labels with both $t_{\text{even}}$ and $t_{\text{odd}}$, must appear in the assembly.

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.