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Inverse protein folding in 3D hexagonal prism lattice under HPC model

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Abstract

The inverse protein folding problem is that of designing an amino acid sequence which has a prescribed native protein fold. This problem arises in drug design where a particular structure is necessary to ensure proper protein-protein interactions. In Gupta et al. (2007), a tubular structures for 3D hexagonal prism lattice were introduced and their stability was formally proved for simple instances under the HP model of Dill. In this paper, we generalize the design of tubular structures to allow for much larger variety of designable structures by allowing branching of tubes. Our generalized design could be used to roughly approximate given 3D shapes in the considered lattice. Although the generalized tubular structures are not stable under the HP model we can prove that a simple instance of generalized tubular structures is structurally stable (folds into designed shape) under a refined version of HP model, called HPC model. We conjecture that there is a way how to choose which hydrophobic monomers are cysteines in generalized tubular structures such the designed proteins are structurally stable under the HPC model.

1 Introduction

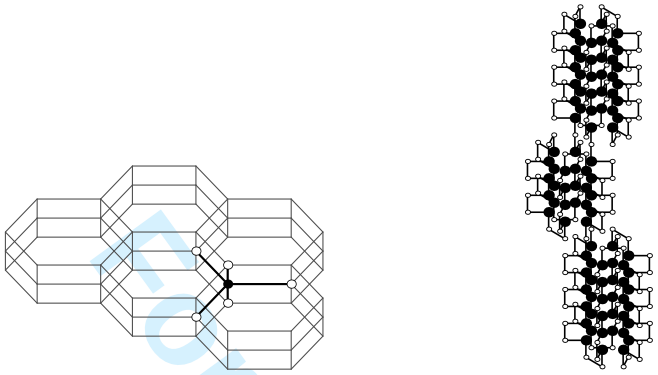
It has long been known that protein interactions depend on their native three-dimensional fold and understanding the processes and determining these folds is a long standing problem in molecular biology. The most significant force acting on protein folding are hydrophobic interactions (see Dill (1990) for details). This led Dill to introduce the *Hydrophobic-Polar model* Dill (1985). Here the 20 amino acids from which proteins are formed are replaced by two types of monomers: hydrophobic or polar, depending on their affinity to water. To simplify the problem, the protein is laid out on vertices of a lattice with each monomer occupying exactly one vertex and neighboring monomers occupy neighboring vertices. The free energy is minimized when the maximum number of non-consecutive hydrophobic monomers are adjacent in the lattice. Therefore, the "native" folds are those with the maximum number of such HH contacts. Even though the HP model is the simplest model of the protein folding process, computationally it is an NP-hard problem, cf. Crescenzi et al. (1998) for two- and Berger and Leighton (1998) for three-dimensional square lattices.

Another significant force in the folding process of the proteins are disulfide bridges between two cysteine monomers which play an important role in the stability of the protein structure Jaenicke (1991). In Hadj Khodabakhshi et al. (2008) we extended the HP model by considering a third type of monomers, cysteines, and incorporating disulfide bridges between two cysteines into the energy model. This model is called the hydrophobic-polar-cysteine (HPC) model. The cysteine monomers in the HPC model act as hydrophobic Naganoa et al. (1999), but in addition two neighboring cysteines can form a disulfide bridge to further reduce the energy of the fold.

In many applications such as drug design, we are interested in the complement problem to protein folding: *inverse protein folding* or *protein design*. A major challenge in protein design is to avoid proteins that have multiple native folds. We say that a protein is *stable* if its native fold is unique. Furthermore, we say that a protein is *structurally stable* if all its native folds define the same mapping from the set vertices of the lattices to the set of amino acids, i.e., if all native folds appear to be identical and only differ by the peptide connections. In Gupta et al. (2005) a new version of the inverse protein folding problem was considered:

*These authors contributed equally.

instead of a target fold, a target structure (a connected set of lattice vertices) is given, and the goal is to design a sequence which would (preferably uniquely) fold into a structure (picked from a rich class of "constructible" structures) "close" to the target structure. The 2D square lattice was used and it was shown that all designed proteins fold into corresponding constructible structures. It was also "formally" shown that the proteins for the simplest (but arbitrary long) constructible structures fold uniquely, and conjectured that the same holds for all constructible structures. Design of stable proteins of arbitrary lengths in the HP model was also studied in Aichholzer et al. (2003) (for 2D square lattice) and in Li et al. (2005) (for 2D triangular lattice), motivated by a popular paper of Hayes (1998).



(a) (b)

Figure 1: An example of (a) hexagonal prism lattice; (b) a tubular structure built with 3 tubes. Hydrophobic (polar) monomers are depicted with black (white) beads.

In Gupta et al. (2007), the 3D lattice (hexagonal prism lattice, cf. Figure 1(a)) was used to design a class of tubular structures and their corresponding proteins. It was shown that each protein folds into the corresponding tubular structure, and that the proteins for the smallest tubular structures (with up to two tubes) are structurally stable under the HP model, however in the two tubes case, the paper missed one case in which protein for the tubular structure with two tubes fold into a very similar structure, and hence, it is not completely structurally stable. An example, of a tubular structure is shown in Figure 1(b). The shortcoming of this design is that it only allows to chain tube in linear fashion which severely limits ability of design to approximate given shapes.

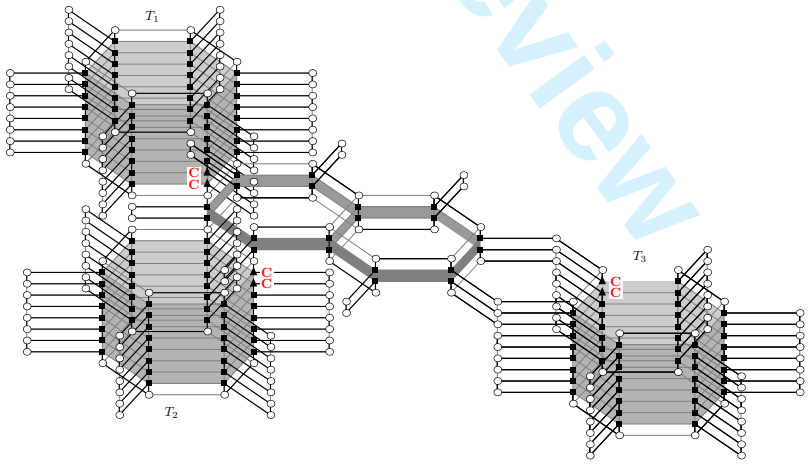


Figure 2: An example of a generalized tubular structure showing the ability to branch (on the left). Polar, hydrophobic and cysteine monomers are depicted as empty circles, squares and triangles, respectively. Hydrophobic cores of 3 tubes and a connector are highlighted.

In this paper, we generalize the design introduced in Gupta et al. (2007) by adding a new building

block: a "*connector*". The hydrophobic core of the connector consists of 2 layers of two adjacent hexagons. The connector can be attached to 4 tubes (one per top/bottom of each hexagon). We call these structures *generalized tubular structures*. An example, with 3 tubes attached to the connector is shown in Figure 2. Such design is sufficiently robust to roughly approximate any given shape.

We show that a generalized tubular structure is one of the native folds of its protein under HPC model. We conjecture that the proteins of the generalized tubular structures are structurally stable, i.e., their proteins fold uniquely into designed structures. We are able to prove this formally for infinite subclass of the simple structures (consisting of one connector and three tubes, cf. Figure 2) under the assumption that each of the three tubes is sufficiently long. In addition, similar to Gupta et al. (2007), we assume that our proteins are closed chains of monomer, a similar assumption as used in Aichholzer et al. (2003), i.e., that the beginning and the end of the sequence are adjacent in the lattice. Note that generalized tubular structures from this subclass are not structurally stable under the HP model, thus our results show that using disulfide bridges in our designs helps to stabilize them.

Despite the tremendous amount of work on protein design for 2D lattices, as far as we know, this is the first general design of arbitrary long stable proteins for the 3D lattice. Given that 3D is the realistic setting, we believe that this work could eventually help in designing proteins with applications to drug design and nanotechnology.

2 Preliminaries

In this section we will review the HPC model and introduce some terminology used in the paper.

2.1 Hydrophobic-polar-cysteine model

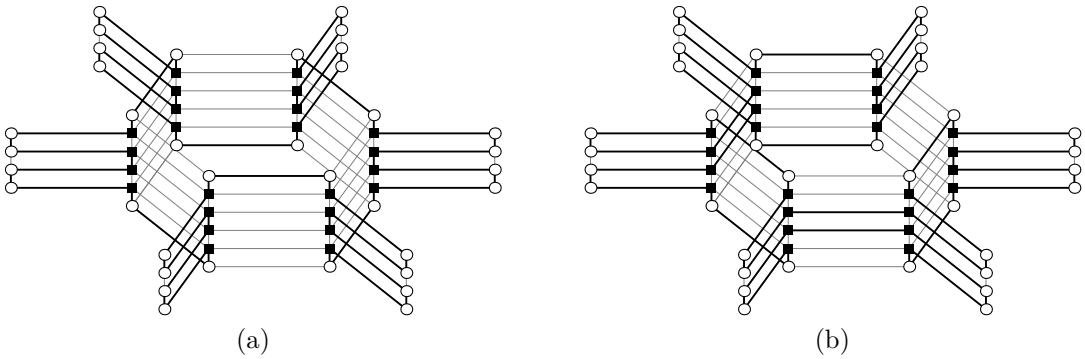
In HPC model, proteins are chains of monomers where each monomer is either hydrophobic-none-cysteine, cysteine or polar. Such a chain is represented as a string $p = p_1 p_2 \dots p_{|p|}$ in $\{0, 1, 2\}^*$, where "0" represents a polar monomer (depicted in figures as empty circles), "1" a hydrophobic-none-cysteine (depicted as black squares) and "2" a cysteine monomer (depicted as black triangles). We use H to represent a monomer which could be either 1 or 2 (depicted in figures as a black circle). The proteins are folded onto the regular lattice. A *fold* of a protein p is embedding of a path of length n into lattice.

In our 3D HPC model we use the hexagonal prism lattice as a lattice structure. The vertices adjacent to a vertex are called the neighbors of that vertex. As depicted in Figure 1(a), each vertex has 5 neighbors: 3 horizontal neighbors lying in the same hexagonal grid and 2 vertical neighbors lying above and bellow the vertex in the parallel hexagonal grids.

A protein will fold into a conformation with the minimum free energy, also called a *native fold*. The energy function in the HPC model consists of two parts: *hydrophobic interactions* and *disulfide bridges*. The hydrophobic monomers which are not consecutive in the protein but are adjacent in the lattice form (*contacts*). Each contact contributes with -1 to the total energy. The cysteines act as hydrophobic monomers in this part of energy function. In addition to hydrophobic interactions a pair of cysteines which are not consecutive in the protein but are adjacent in the lattice form disulfide bridges and further reduce the energy of the fold. Unlike the hydrophobic interactions in which a hydrophobic monomer can take part in several contacts, a cysteine can only participate in one disulfide bridge. Therefore, the number of disulfide bridges contributing in the energy of fold is equal to the number of pairs in the maximum matching in the graph of potential disulfide bridges. Each disulfide bridge contributes with -1 to the total energy. Hence, a fold with the lowest free energy corresponds to a fold with the largest number of HH contacts and disulfide bridges.

2.2 Stability

Note that there might be several native folds for a given protein. A protein with a unique native fold is called a *stable* protein. Every protein and its fold define a mapping from the lattice vertices to the set $\{0, 1, 2, W\}$, where W represents "water" or an empty unoccupied position. We say that two folds of the same protein are *similar* if they define the same mapping. If all native folds of a given protein are similar to each other, then the protein is called *structurally stable*. Note that all native folds of a structurally stable protein have completely same shape (from outside their appear as a same fold). For instance, the string $t = (0100110010)^6$



(a) (b)

Figure 3: Two native folds of the substring $t = (0100110010)^6$. These two folds are similar.

is structurally stable, but not stable. Figure 3 depicts all two native folds of this string. It is easy to see that the mappings defined by t and its two folds are identical, i.e., the folds are similar.

2.3 Terminology

A lattice vertex containing an $\mathsf{X} \in \{0, 1, 2\}$ monomer is called an X-vertex. An H-vertex is either a 1-vertex or a 2-vertex. A neighbor of a vertex v which is an X-vertex is called X-neighbor.

Consider a fold F. A path in F is a sequence of vertices $(x_1, x_2, \ldots, x_k)$ such that consecutive vertices are connected by peptide bonds. We say that F contains an occurrence of substring $w_1, w_2, \ldots, w_k$ if there is a path $(x_1, x_2, \ldots, x_k)$ in F such that x_i is a w_i-vertex.

We number hexagonal grids of the lattice (also referred to as *planes*) with integer numbers, and denote the i-th grid by H_i. Consider vertex $x \in H_i$. We denote the vertical neighbor of x in H_{i+1} (above x) by x^1, and recursively, the vertical neighbor of x^j in H_{i+j+1} by x^{j+1}. Similarly, we denote the neighbor of x in H_{i-1} by x^{-1}, and the neighbor of x^{-j} in H_{i-j-1} by x^{-j-1}.

Let G_x be the graph of all H-vertices in H_i which are reachable from x by a path of H-vertices in H_i. Let G be a set of vertices in H_i. Then for $j \geq 1$, let G^j be the graph of all vertices in H_{i+j} which have a neighbor in G^{j-1}, and G^{-j} be the graph of all vertices in H_{i-j} which have a neighbor in G^{-j+1}, i.e., G^j and G^{-1}, $j \neq 0$, are vertical copies of the set G.

Note that G_x is a planar graph (as H_i is as well). The degree of a vertex in G_x is called a *plane degree*. Let B_x be the boundary cycle of G_x, i.e., the set of vertices of G_x which lie on the outer face of G_x. A *component* in a fold F is a maximal set of H-vertices for which there is a path of H-vertices between any pair of them.

Let C be a component that lies in the planes H_{j+1} to H_{j+r}. Let layer C_i be a graph of all vertices of C in plane H_{j+i+1}. We say that layers C_i and C_k are the same if $C_i^{k-i} = C_k$, i.e, C_k is a copy of C_i. When we say that we are comparing layers C_i and C_j of component C, we mean comparing the sets C_i and C_j^{i-j}. For example, when we say C_i is identical to (respectively, a subset of) C_j we mean whether C_i is identical to (respectively, a subset of) C_j^{i-j}, and we write simply $C_i = C_j$ (respectively, $C_i \subseteq C_j$). The plane containing C_i will be denoted by $H(C_i)$.

2.4 Saturated folds

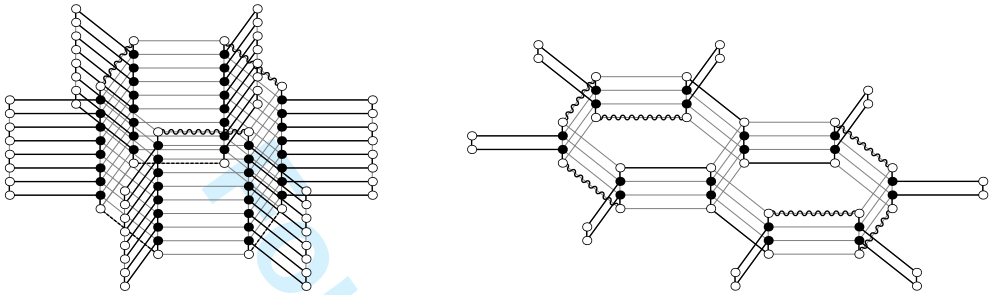
The proteins used in Gupta et al. (2005) and the proteins we will use in our design have a special property. The number of possible contacts and disulfide brides of their native folds is maximal with respect to the number of hydrophobic "1" and cysteine "2" monomers contained in the protein. The following useful observation characterizes native folds of such proteins.

Observation 1 (Saturated folds). *Let $p \in 0\{0,1,2\}^*0$ be a protein, and F be the fold of p. If for every* H*-vertex v, three out of five edges incident with v are contacts and in addition if v is a cysteine it belongs to a maximum matching in the graph of potential disulfide bridges, then (a) F is a native fold of p; and (b) any*

other native fold of p satisfies these properties. We will call a fold satisfying these properties a saturated *fold.*

The proof of the observation follows by a simple argument that any hydrophobic vertex v can have at most three contacts since it is connected to exactly two neighbors with a peptide bond and furthermore any cysteine monomer can be involved in at most one disulfide bridge. Note that not every protein has a saturated fold.

3 Generalized tubular structures and their proteins



(a) (b)

Figure 4: (a) Illustration of a tube with a hydrophobic core of height 8 — the wavy lines at the top and dashed lines at the bottom represents loops. (b) Illustration of a connector.

The first basic building block of our *generalized tubular structures* is a *tube*, depicted in Figure 4(a). Tubes were the only building block of tubular structures introduced in Gupta et al. (2007). A tube consists of 6 identical "alpha helix"-like subfolds of the substring $p_n = (\mathsf{H00H})^n$ forming a $2 \times 2n$ vertical zig-zag pattern ("plate").

The plates are connected to each other with 6 short loops (3 at the top and 3 at the bottom), each consisting of only two polar monomers. Thus, the hydrophobic core is completely surrounded by polar monomers, i.e., the fold is saturated. The complete protein string for the tube is $t_n = (\mathsf{0}p_n\mathsf{0})^6$. We assign the first and the second H monomer of one of the plates of each tube to cysteine monomers 2. We represent the fold of t_n by $\mathcal{T}_n$. The height of the hydrophobic core of the tube $\mathcal{T}_n$ is $2n$.

The second building block of our generalized tubular structures is a *connector*, depicted in Figure 4(b). A connector can be formed by overlapping two very short tubes (with height of hydrophobic core 2). Two tubes or a tube and a connector can be connected to one protein structure in two ways as follows. First, one top loop of the first tube is overlapped with a bottom loop of the second tube/connector, vice versa, and the peptide bonds between two polar monomers of each loop are disconnected. This way of connecting two components is called *vertical* connection. Tubes T_1 and T_2 in Figure 2 are vertically connected to the connector. In the second way, called *horizontal* connection, the tubes or the tube and the connector are placed beside each other such that they have H-vertices in exactly one common plane H_i and exactly two H-vertices of the first component are connected to two H-vertices of the other component each through one 0-vertex. Tube T_3 in Figure 2 is horizontally attached to the connector. Repetitively, connecting tubes and connectors (such that no space violation occurs) we obtain the class of *generalized tubular structures*. We choose to vertically or horizontally connect a tube to a component in a generalized tubular structure such that no pair of H-vertices in the same plane and in middle layers of different tubes are at distant three of each other. Since, there is no substring 000 in the protein of any generalized tubular structure, this condition ensures that the tubes in a generalized tubular structures do not directly connect to each other through the H-vertices in their middle layers. This will greatly simplifies the stability proof of the structures.

Since, the folds of tubular structures are saturated, by Observation 1, they are native folds to corresponding proteins (which can be easily reconstructed from the folds).

4 Stability of generalized tubular structures

In what follows we will show that the protein of one basic generalized tubular structure: the structure built from one connector and three tubes, cf. Figure 2, is structurally stable. We will assume that three tubes $\mathcal{T}_{k_1}, \mathcal{T}_{k_2}, \mathcal{T}_{k_3}$ used to construct this structure are sufficiently long. In particular, we will assume that $k_1, k_2, k_3 \geq 712$. We conjecture that this structure is structurally stable also for other values of k_1, k_2, k_3 and that all generalized tubular structures are structurally stable. Let q be the protein string of this structure and Q be its original fold.

Definition 1 (sparse protein). We say that a protein is *sparse* if does not contain HHH as a substring and does not start or end with H.

4.1 Types of H-vertices

Let F be a saturated fold of a sparse protein. Then each H-vertex has exactly three contacts, i.e., it has at least three H-neighbors and the remaining two neighbors are connected (via a peptide bond) and at most one of the two is an H-vertex. We can classify every H-vertex x of S to one of the five types based on the position of its 0-neighbor(s), cf. Figure 5:

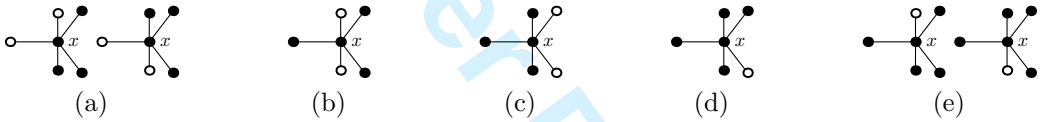
(a) vh-type: x has one vertical 0-neighbor (on top or below) and one horizontal 0-neighbor (in the same hexagonal grid);

(b) vv-type: x has two vertical 0-neighbors;

(c) hh-type: x has two horizontal 0-neighbors;

(d) h-type: x has one horizontal 0-neighbor;

(e) v-type: x has one vertical 0-neighbor.



(a) (b) (c) (d) (e)

Figure 5: Five types of possible neighborhood of an H-vertex x: S-vertices: (a) vh, (b) vv, (c) hh; and D-vertices: (d) h and (e) v.

For every $\mathsf{X} \in \{\mathsf{vv}, \mathsf{hh}, \mathsf{h}, \mathsf{v}\}$ an H-vertex of type X, will be called X-vertex. Furthermore, any H-vertex with two 0-neighbors is called a S-*vertex* and an H-vertex with one 0-neighbor is called a D-*vertex*.

Definition 2 (connections). Let $u, v \in \{0, 1, 2, \mathsf{H}, \mathsf{S}, \mathsf{D}, \mathsf{vv}, \mathsf{hh}, \mathsf{h}, \mathsf{v}\}$ and $s \in \{0, 1, 2, \mathsf{H}\}^+$. We say that two vertices x and y are *s-connected* if there is a path $x, v_1, v_2, \ldots, v_k, y$ such that v_i is an s_i-vertex. If x is a u-vertex and y is a v-vertex, this path is called an *usv-connection*. If the end points x and y are H-vertices and belong to two different components, we say that these components are *usv-connected*. If $s = 00$ and $u, v \neq 0$, we will shorten this notation as $(u\backslash v)$-connection. In particular, we will be interested in H0H-connections and (S\h)-connections.

A *usv*-connection with end points x and y is called *internal*, if x and y are in the same component, and otherwise it is called *external*. We say that two *usv*-connections with end points at x, y and x', y', respectively, are *parallel* if x (y) is directly above/below x' (y'), i.e., $x' = x^i$ and $y' = y^j$, for some integers i, j, and all vertices between x and x' (y and y') are H-vertices. Note that it is also possible that x and y' are u-vertices and x' and y are v-vertices.

We have the following observations:

Observation 2. *Let F be an arbitrary saturated fold of q. Then F contains 6* H0H*-connections,* 52 S*-vertices, the number of* D*-vertices is 4 modulo 6 and it contains* 36 (S\D)*-connections. F does not contain* HHH*,* 000*,* H0H0H *and* H0HH*, but it does contain one occurrence of* 20100101*.*

Observation 3. *Let F be a saturated fold of a sparse protein. Then every* H*-vertex of F is either a* vh*-vertex,* vv*-vertex,* hh*-vertex,* h*-vertex or* v*-vertex. Furthermore, any neighboring* 0*-vertex and* H*-vertex are connected by a peptide bond.*

Claim 1. *Let F be a saturated fold of a sparse protein with no* H0HH *as a substring. Then no* v*-vertex in F can connect directly to an* h*-vertex.*

Proof. Consider a v-vertex x. Without loss of generality assume that its 0-neighbor is x^1. Assume to the contrary that x connects to an h-vertex. Two cases are possible: first, x connects to its horizontal h-neighbor z cf., Figure 6(a). Then z^1, x^1, x, z form the substring H0HH, a contradiction. Second, x connects x^{-1} which is an h-vertex. Let z be the horizontal 0-neighbor of x^{-1}. Then z^1, z, x^{-1}, x form the substring H0HH, a contradiction cf., Figure 6(b). □

Claim 2. *Let F be a saturated fold of a sparse protein. No* v*-vertex can connect to an* h*-vertex via two* 0*-vertices.*

Proof. Consider a v-vertex x. Without loss of generality assume that its 0-neighbor is x^1. Assume to the contrary that x^1 connects to an h-vertex via one 0-vertex y. If y is a horizontal neighbor of x^1 then it would connect down to an a vertex which is not an h-vertex. Hence, $y = x^2$. Furthermore, x^2 should connect to an h-vertex, hence it cannot connect to x^3. Therefore it must connect to one of its horizontal neighbor z. Since, z is an h-vertex, z^{-1} is an H-vertex. However, this a contradiction, as x^1 would have to connect to three vertices: x, x^2 and z^{-1} Figure 6(c). □

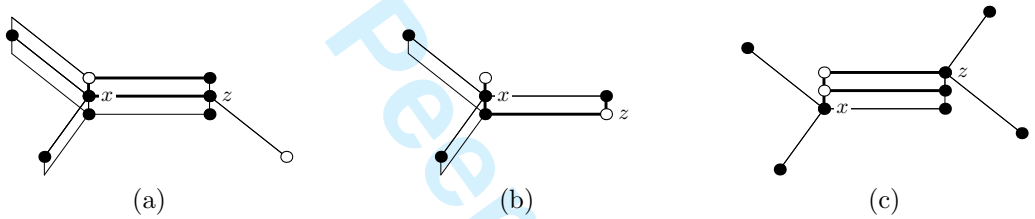


Figure 6: Case analysis showing that a vh-vertex cannot directly (a) and (b); or via two 0-vertices (c) connect to an h-vertex

The above two claims imply the following lemma.

Lemma 1. *Let F be a saturated fold of a sparse protein with no* H0HH *as a substring. Any occurrence of substring* $(00HH)^k$ *in F contains either only* v*-vertices or only* h*-vertices.*

4.2 Types of components

In this section we study all possible components that can arise in saturated folds of q. We first classify all components to three categories and then study which of these can appear in saturated folds of q.

Let F be a saturated fold of a sparse protein and C a component in F. Assume that C lies in the planes $H_s, \ldots, H_e$. Note that any H-vertex of plane degree one in the first or last layer of C is adjacent to at least three 0-vertices, a contradiction. Hence, we have the following observation.

Observation 4. *Let F be a saturated fold of a sparse protein and let C be a component in F. Then all vertices of the first or last layer of C have plane degree* 2 *or* 3*.*

The following definition defines several types of components.

Definition 3 (tube, simple tube, 2-layer component, wall, and complex component). A *tube* is a component such that all its layers are identical and each layer contains only vertices of plane degree two (a cycle). A *simple tube* is a tube with only one hexagon in each layer. A *2-layer component* is a component with two identical layers which have no vertex with plane degree 1 and at least one vertex with plane degree 3. A *wall* is a component such that all its layers are identical and each layer is a single path. Finally, a *complex component* is a component C such that there is i for which C_i and C_{i+1} are different.

We have the following observations.

Observation 5. *Any component C in a saturated fold of a protein is one of the following three types: a tube, a 2-layer component or a complex component.*

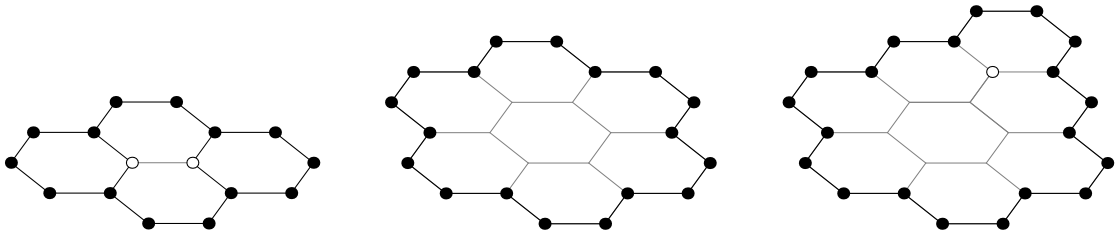


Figure 7: One layer of (a) the smallest non-simple tube; (b) the smallest non-simple tube without occurrences of H0H; and (c) the smallest non-simple tube with one occurrence of H0H per layer.

Observation 6. *Let F be a saturated fold of a sparse protein. If F contains a tube then the height (number of layers) of this tube is at least 2. One layer of the smallest non-simple tube is depicted in Figure 7(a). It contains two occurrences of* H0H *per layer, i.e., at least 4 such occurrences. One layer of the smallest non-simple tube with no occurrences of* H0H *is depicted in Figure 7(b). One layer of the smallest tube with one occurrence of* H0H *per layer is depicted in Figure 7(c).*

4.3 Different types of complex components

In what follows we further classify different types of complex components which can occur in saturated folds of sparse proteins with at most six occurrences of substring H0H.

4.3.1 Complex components with a vv-vertex

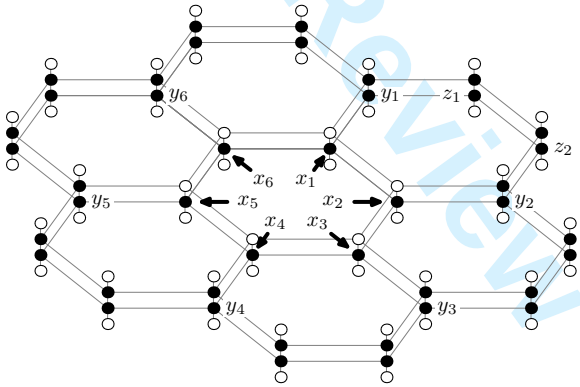


Figure 8: Part of a complex component with a vv-vertex. The arrows are pointing at six vv-vertices.

Lemma 2. *Let F be a saturated fold of a sparse protein with no occurrences of substrings* H0HH *and* H0H0H *and at most six occurrences of substring* H0H*. Consider a complex component C of F containing a* vv*-vertex. Then C has 6* vv*-vertices forming a hexagon, lies in two layers which are almost identical, except for the six* vv*-vertices which are replaced with* 0*-vertices in the other layer, and neither layer contains a vertex of plane degree* 1*. We will call such a complex component, a* vv-component*. A* vv*-component contains 6 occurrences of* H0H*.*

Proof. Any vv-vertex must be adjacent to at least two other vv-vertices in its plane, otherwise, either there is a 0-vertex connected to three H-vertices (with a peptide bond), or we get a substring H0H0H which cannot occur in F. Therefore, any set of vv-vertices in a plane forms a graph with no vertices of plane degree 1. Each vv-vertex on the boundary of this graph is adjacent to one non-vv-vertex which creates a distinct H0H substring. Since there are only 6 occurrences of H0H in F, the boundary of this graph must be a hexagon, i.e., C contains exactly 6 vv-vertices $x_1, \ldots, x_6$ located on a single hexagon, cf. Figure 8. Furthermore, C does not contain a vertex with plane degree 1. Assume to the contrary that v is a vertex with plane degree 1 and let k be the smallest number such that v^k is a vertex with plane degree more than 1 (note that such a k exists). Let w be a horizontal H-neighbor of v^k. Now, the path (w, w^{-1}, v^{k-1}) is an H0H-connection which is different from the H0H-connections containing the vv-vertex of F, a contradiction.

For $i = 1, \ldots, 6$, let y_i be the non-vv horizontal neighbor of x_i. Consider y_1. One of its vertical neighbor is an H-vertex while the other is a 0-vertex, cf. Figure 8. Without loss of generality assume y_1^1 is an H-vertex. Let z_1 be the horizontal neighbor of y_1 which is closer to y_2. Since C does not contain any vertex of plane degree 1, all the horizontal neighbors of y_1^1 except x_1^1, are H-vertices. In addition, y_1^2 must be a 0-vertex otherwise, F would contain the substring H0HH, a contradiction. It follows that z_1 is an H-vertex and z_1^{-1} and z_1^2 are 0-vertices otherwise, we get additional H0H-connections, a contradiction.

Next, we show that y_2^1 is an H-vertex. Let z_2 be the common neighbor of z_1 and y_2. Clearly, z_2 is an H-vertex otherwise we get another H0H-connection, a contradiction. Similarly, z_2^{-1} and z_2^2 are 0-vertices and z_2^1 is an H-vertex. It follows that y_2^1 is an H-vertex. By similar arguments, we can show that for every $i = 1, \ldots, 6$, y_i^1 is an H-vertex and y_i^{-1} and y_i^2 are 0-vertices. Since there is no other occurrence of H0H in F, it is easy to see that the whole component lies in two layers (the layers containing y_i's and y_i^1's) which are almost identical with exception that 6 vv-vertices in lower layer replaced with 0-vertices in the upper layer. □

Note that a vv-component is essentially a 2-layer component which is missing vertices of one hexagon in one of the two layers.

4.3.2 Complex components without a vv-vertex

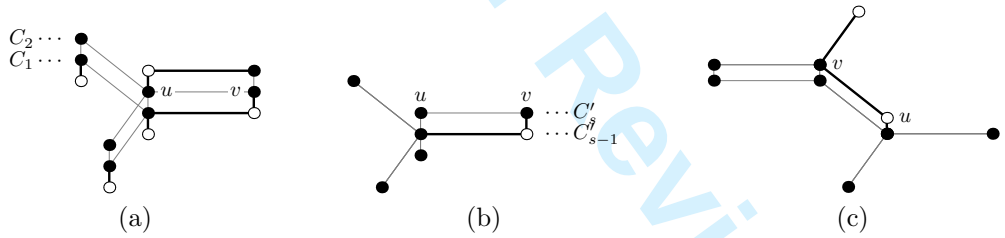


Figure 9: Analysis of a complex component without a vv-vertex: (a) the case in which $C'_2 \neq C_1$; (b) the case in which C'_i is not a subset of C_1; (c) the case when C'_s is not a subset of $V^{2,2,2}$.

Lemma 3. *Let F be a saturated fold of a sparse protein with no* H0HH *as a substring. Let C be a complex component of F without a* vv*-vertex and $C_1, \ldots, C_r$ its layers. Let $V^{2,2,2}$ be the set of all* H*-vertices in F with plane degree* 2 *such that both its horizontal* H*-neighbors have plane degree* 2 *as well.*

(a) For $k \geq 1$, let C'_k be a subset of C_k consisting of components of C_k which are intersecting C_1. Let s be the smallest integer such that layer C'_s is different from C_1. Then $s > 2$ and C'_s is a collection of paths where each path is a subset of $C_1 \cap V^{2,2,2}$.

(b) For $k \leq r$, let C''_k be a subset of C_k consisting of components of C_k which are intersecting C_r. Let e be the largest integer such that layer C''_e is different from C_r. Then $e < r - 1$ and C''_e is a collection of paths where each path is a subset of $C_r \cap V^{2,2,2}$.

Proof. We prove only part (a) of the lemma, part (b) follows by symmetry. Since there is no vv-vertex in C, C_2 and hence, also C'_2 is a superset of the C_1. We show that these two layers are identical. To the contrary

assume that C'_2 contains a vertex w such that its vertical neighbor in the plane $H(C_1)$ is a 0-vertex. Since C'_2 is intersecting C_1, there must be a shortest path connecting w to some vertex u of C^1_1. Note that $u \in C'_2$ and $u^{-1} \in C_1$. Let v be the neighbor of u on this paths, i.e., v is an H-vertex in C_2 and v^{-1} is a 0-vertex. Since, the plane degree of u^{-1} is at least 2, its horizontal neighbors other than v^{-1} are H-vertices. Since, $C_1 \subseteq C'_2$, all horizontal neighbors of u are H-vertices, i.e., u is a v-vertex. Therefore, u^1 is a 0-vertex. Furthermore, since there is no vv-vertex in F, v^1 is an H-vertex, cf. Figure 9(a). Since, u is a D-vertex, it is connected to one of its H-neighbors, say z. Then, v^1, u^1, u, z form the substring H0HH, a contradiction. Hence, $C_1 = C'_2$.

Let s be the smallest integer such that C'_s is different from C_1. Since $C_1 = C'_2$, it follows that $s > 2$. Next, we show that C'_s is a subset of $C_1 = C'_{s-1}$. Assume the contrary. Since C'_s is intersecting $C_1 = C'_{s-1}$, there exists an H-vertex v and its horizontal H-neighbor u in C'_s such that v^{-1} is a 0-vertex and u^{-1} is a H-vertex in C'_{s-1}. Since $C'_{s-1} = C'_{s-2} = C_1$, the plane degree of u^{-1} is 2 and u^{-2} is an H-vertex, cf. Figure 9(b). Hence, u^{-1} is a D-vertex, i.e., it is connected to some H-vertex z. Then v, v^{-1}, u^{-1}, z form the substring H0HH, a contradiction.

Finally, note that any vertex with plane degree 3 in C'_{s-1} must have a 0-neighbor in the plane $H(C_s)$, as otherwise it would have five H-neighbors. Since, C'_s is a subset of $C_1 \cap V^2$, where V^2 is the set of all H-vertices in F with plane degree two, C'_s is a collection of paths.

Finally, let us prove that each path in C'_s lies in $V^{2,2,2}$. Assume the contrary. Then the end point v of such a path in C'_s has a 0-neighbor u such that u^{-1} is an H-vertex of plane degree 3 in C'_{s-1}. Hence, u^{-1} is a v-vertex and we have an occurrence of H0HH (cf. Figure 9(c)), a contradiction. □

Lemma 4. *Let F be a saturated fold of a sparse protein with no occurrences of the substring* H0HH*, and at most six occurrences of the substring* H0H*. Let C be a complex component of F without a* vv*-vertex and $C_1, \ldots, C_r$ be its layers. Let $\bar{s} > 2$ ($\bar{e} < r-1$) be the smallest (largest) integer such that $C_{\bar{s}}$ ($C_{\bar{e}}$) is different from C_1 (C_r). Then both $C_{\bar{s}}$ and $C_{\bar{e}}$ contain a single path, and each of the layers $C_1, \ldots, C_{\bar{s}}, C_{\bar{e}}, \ldots, C_r$ is connected. Furthermore, each complex component creates at least four occurrences of the substring* H0H *in F, two between layers $C_{\bar{s}-1}$ and $C_{\bar{s}}$ and other two between layers $C_{\bar{e}}$ and $C_{\bar{e}+1}$.*

Proof. Let C'_k, C''_k be the sets and s, e the integers defined in Lemma 3. By this lemma, both C'_s and C''_e are collections of paths. Each paths in C'_s and C''_e creates two new occurrences of substring H0H. Therefore, the total number of paths in C'_s and C''_e is either 2 or 3.

First, assume that C'_s and C''_e contain 2 paths in total. It is enough to show that for every $k = 2, \ldots, s$, $C'_k = C_k$ and for every $k = e, \ldots, r-1$, $C''_k = C_k$. Assume that there is $l \in \{2, \ldots, s\}$ such that $C'_l \neq C_l$ and assume that l is the smallest such integer. Then C_l contains another component K which does not intersect $C_1 = C'_l$. Note that we can apply Lemma 3 on K as well, i.e., there will be a level $l' > l+1$ such that all components of $C_{l'}$ intersecting K are paths. Since, each such paths will create 2 occurrences of H0H, there is only one such path P. Note that there is no other occurrence of H0H in F. It is easy to see that for all $s < k < e$, $C'_k = C_s$, as any change would introduce a new occurrence of H0H. Similarly, for any $l' < k < e$, there is only one component of C_k intersecting K, P. Now, the layer C_{e-1} contains two paths and C_e only one path. Thus, the change from C_{e-1} to C_e introduces new occurrences of H0H, a contradiction. Hence, for every $k = 2, \ldots, s$, $C'_k = C_k$ and for every $k = e, \ldots, r-1$, $C''_k = C_k$. This implies that $\bar{s} = s$ and $\bar{e} = e$. The lemma follows.

Second, assume that C'_s and C''_e contain 3 paths in total. Without loss of generality assume that C'_s contains 2 paths and C''_e has only 1 path. This will create 6 occurrences of H0H in F. Therefore, as before, C_{e-1} contains two paths and C_e only one path, a contradiction. □

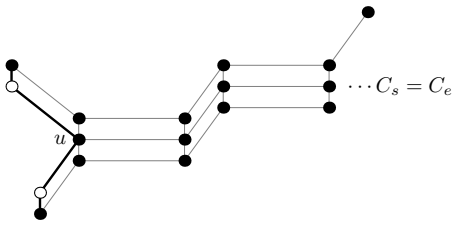


Figure 10: A complex component: the case when layers C_s and C_e are identical.

Observation 7. *Let F be a saturated fold of a sparse protein with no occurrences of the substrings* H0HH *and* H0H0H*, and at most six occurrences of the substring* H0H*. Let C be a complex component without a* vv*-vertex. Let $s > 2$ $(e < r - 1)$ be the smallest (largest) integer such that C_s (C_e) is different from C_1 (C_r). Then $s \neq e$, i.e, the middle part of a complex component without a* vv*-vertex (layers $C_s, \ldots, C_e$) contains at least 4* S*-vertices..*

Proof. First, notice that if $s = e$ then the end point of the path u in $C_s = C_e$ belongs to two different occurrences of H0H. If these two occurrences share a 0-vertex v then v connects to three vertices, a contradiction. Otherwise, we have an occurrence of substring H0H0H, cf. Figure 10, again a contradiction. □

4.3.3 Basic complex component

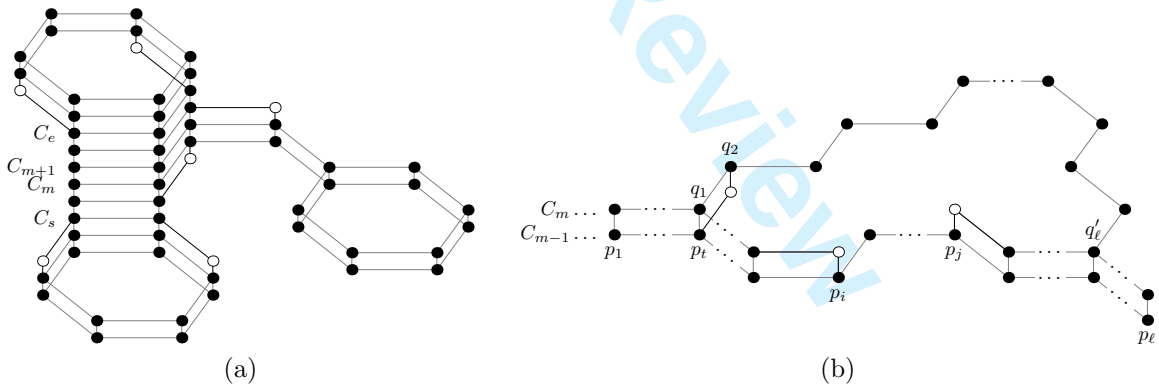
Definition 4 (basic complex component). Let F be a saturated fold of a sparse protein with no H0HH as a substring. Let C be a complex component of F without a vv-vertex with layers $C_1, \ldots, C_r$. Let s be the smallest integer such that C_s is different from C_1 and let e be the largest integer such that C_e is different from C_r. If C_s is a path and for any $i \in s + 1, \ldots, e$, C_i is identical to C_s then we call C a *basic complex component.*

Note that a basic complex component consists of three parts stack vertically on each other: (1) a tube or 2-layer component; (2) a wall; and (3) a tube or 2-layer component.

Observation 8. *Let F be a saturated fold of a sparse protein with no* H0HH *as a substring. Any basic complex component of F contains at least 20* S*-vertices (the lower and upper part at least 8 each and the wall at least 4) and at least 4 occurrences of substring* H0H*.*

4.3.4 Appendix components

In this subsection, we show that if a complex component C without vv-vertices is not basic, then its layers change exactly four times, i.e., it consists of five parts stacked on top of each other: (1) a 2-layer component or a tube; (2) a wall; (3) a pseudo 2-layer component with exactly one vertex with plane degree 1 in each of two layers; (4) another wall; and (5) a 2-layer component or a tube. The part in the middle (3) will be called an *appendix*, and such a complex component will be called an *appendix component.* An example of an appendix component is in Figure 11(a). Let us start with the formal definition of an appendix component.



(a) (b)

Figure 11: (a) An example of an appendix component and the six occurrences of H0H contained in it. (b) Illustration what happens if C_{m-1} is not a subset of C_m.

Definition 5 (appendix component). Let F be a saturated fold of a sparse protein with no occurrence of the substring H0HH. Let C be a complex component of F without a vv-vertex with layers $C_1, \ldots, C_r$. Let s be the smallest integer such that C_s is different from C_1 and let e be the largest integer such that C_e is different from C_r. Assume that both C_s and C_e contain only one path, and that there is an integer $s < m < e - 1$ such that $C_s = C_{s+1} = \cdots = C_{m-1}$, $C_m = C_{m+1}$, $C_{m+2} = C_{m+3} = \cdots = C_s$, and either C_s is a subset of C_e or C_e is a subset of C_s and both of them are subsets of C_m. Furthermore, assume that C_m

has exactly one vertex with plane degree 1 and this vertex is an end point of the paths in C_s and C_e. Such a complex component will be called an *appendix component* and the layers C_m and C_{m+1} we be called an *appendix*. Consider a path in C_m (C_{m+1}) starting at the vertex with plane degree 1 and ending before the first vertex with plane degree 3. These paths in C_m and C_{m+1} will be called the *arm* of the appendix.

Note that an appendix without its arm is a proper 2-layer component.

Lemma 5. *Let F be a saturated fold of a sparse protein with no occurrence of the substring* H0HH*, and at most six occurrences of the substring* H0H*. Every non-basic complex component without a* vv*-vertex in F is an appendix component.*

Proof. Consider a complex component C in F without vv-vertices with layers $C_1, \ldots, C_r$. Assume that C is not a basic complex component. Let s (e) be the smallest (largest) integer such that C_s (C_e) is different from C_1 (C_r). By Lemma 4, both C_s and C_e contain only one path. Let m be the smallest integer such that $s < m < e$ and C_m is different from C_s.

First, we will prove that C_s is a subset of C_m. Since, $C_s = C_{m-1}$, C_{m-1} is a path $P = (p_1, \ldots, p_\ell)$. Clearly, p_1^1 and p_ℓ^1 are H-vertices. Assume to the contrary that C_m is not a superset of C_{m-1}. Let p_i (p_j) be the first (last) vertex on path P such that p_i^1 (p_j^1) is a 0-vertex. Clearly, $i \neq j$, hence, we have two new occurrences of H0H. There are no other occurrences of H0H. Therefore, $C_m = C_{m+1} = \cdots = C_e$, i.e., C_m is a path. Thus, there is a path in C_m connecting paths $(p_1^1, \ldots, p_{i-1}^1)$ and $(p_{j+1}^1, \ldots, p_\ell^1)$. Let $(q_1, \ldots, q_{\ell'})$ be a shortest such path. Then $q_1 = p_t^1$ for some $t \in \{1, \ldots, i-1\}$ and q_2^{-1} does not lie on P, i.e., it is a 0-vertex. Then the paths p_t, q_2^{-1}, q_2 forms another occurrence of H0H, a contradiction, cf. Figure 11(b).

Let m' be the largest integer such that $s < m' < e$ and $C_{m'}$ is different from C_e. By symmetry, we have that $C_{m'}$ is a superset of C_e. Obviously, $m \leq m'+1$. We will show that $m \leq m'$, i.e., that there are at least two changes between layers C_s and C_e. Assume to the contrary that $m = m'+1$. Then $C_s = C_{m-1} = C_{m'} \subseteq C_m$ and $C_e = C_{m'+1} = C_m \subseteq C_{m'}$, i.e., $C_m = C_{m'}$. However, this is a contradiction with the fact that C is not a basic complex component, since we have $C_s = \cdots = C_{m-1} = C_{m'} = C_m = C_{m'+1} = \cdots = C_e$.

Since there are at least two changes from layer C_s to layer C_e and each change will introduce at least one new occurrence of H0H, each of the two changes can create only one occurrence of H0H and there are no other changes. Therefore, there is exactly one vertex z in C_m which is a horizontal neighbor of some p_i^1 such that z^{-1} is a 0-vertex. If $i \neq 1, \ell$ then we get an occurrence of H0HH. Hence, C_m extends the copy of path P in the plane $H(C_m)$ at one of its ends. Similarly, $C_{m'}$ extends a copy of the path in layer $C_{m'+1}$ at one of its ends. Furthermore, since there are no other changes $C_m = C_{m+1} = \cdots = C_{m'}$.

It remains to show that $m' = m+1$ and that C_m has exactly one vertex with plane degree 1. The extended part of C_m ($C_{m'}$) does not have a vertex of plane degree one because otherwise it will be an H-vertex with three 0-neighbors. The number of vertices with odd plane degree in C_m ($C_{m'}$) is even. Since, there is only one vertex with plane degree one in C_m ($C_{m'}$), there is an odd number of vertices with plane degree 3, which implies there is at least one such a vertex, say $w \in C_m$. Now, if $m' > m+1$ then $w^1 \in C_{m+1}$ has five H-neighbors, a contradiction. Second, if $m' = m$ then z is a vv-vertex, a contradiction. Hence, $m' = m+1$, i.e, the complex component C has a pseudo 2-layer component between two walls. It follows that C is an appendix component. □

The following observation follows by a careful examination of Figure 11(a).

Observation 9. *Let F be a saturated fold of a sparse protein with no occurrences of the substrings* H0HH*. Let C be an appendix component and C_s, C_m and C_e be the layers after the first, after the second and before the last change, respectively. Then $m \geq s+2$ and $e \geq m+3$. Each wall (layers $C_s, \ldots, C_{m-1}$ and $C_{m+2}, \ldots, C_s$) contains at least 4, the arm of appendix of C at least 4 and the appendix without arm at least 10* S*-vertices. Thus layers $C_s, \ldots, C_e$ contain at least 22* S*-vertices.*

4.4 Counting in one plane

Consider a set S of vertices in a hexagonal plane. Set S naturally induces a graph in the plane in which any two neighboring vertices are connected by an edge. In the following S will represent both the set of vertices and the graph induced by this set. Assume that each vertex of S has a degree at least 2. We say that S is complete if every vertex which lies inside the boundary of S, denoted as $B(S)$, is in S as well. Let $K_{\bigcirc}(S)$

be the number of hexagons which lie inside the boundary $B(S)$, $K_2(S)$ the number of vertices of degree 2 of S and $K_3(S)$ the number of vertices of degree 3. Our goal is to lower bound $K_3(S)$ by some function of $K_2(S)$. We will do that in two steps.

Lemma 6. *Let S be any set of vertices in a hexagonal plane such that each vertex of S has a degree at least 2. We have $K_3(S) \leq 2K_{\hexagon}(S) - 2c$, where c is the number of connected components of S.*

Proof. First, assume that S is a complete 2-connected set. We proceed by induction on $K_{\hexagon}(S)$. If $K_{\hexagon}(S) = 1$ then the lemma trivially holds. There must be a hexagon H in S sharing at least two sides with the boundary $B(S)$ such that all its boundary sides form a single path P. Consider a set S' obtained from S by removing inner vertices of path P. Set S' contains all hexagons contained in S besides H. Thus S' is a complete 2-connected set and the number of hexagons $K_{\hexagon}(S')$ is $K_{\hexagon}(S) - 1$. At the same time, S' must have two vertices of degree 3 less than S (end points of P become vertices of degree 2 and other vertices on P which were removed when constructing S' must have had degree 2). By induction hypothesis, $K_3(S) - 2 = K_3(S') \leq 2K_{\hexagon}(S') - 2 = 2(K_{\hexagon}(S) - 1) - 2$. This implies that $K_3(S) \leq 2K_{\hexagon}(S) - 2$.

Second, assume that S is just a 2-connected set. Let $\bar{S}$ be a set constructed from S by adding all vertices which lies inside the boundary $B(S)$. Note that $B(\bar{S}) = B(S)$ and $\bar{S}$ is complete. Furthermore, the number of vertices of degree 3 of $\bar{S}$ could only increase when adding vertices to S. Therefore, $K_3(S) \leq K_3(\bar{S}) \leq 2K_{\hexagon}(\bar{S}) - 2 = 2K_{\hexagon}(S) - 2$.

Third, assume that S is connected and let $S_1, \ldots, S_l$ be 2-connected components of S. Contracting every 2-connected component to a single vertex we obtain a tree T. Every vertex of T of degree 1 or higher than 3 must be a contracted vertex and the number of contracted vertices is l. Let n_d be the number of all vertices of degree d and let n'_d the number of all contracted vertices of degree d. Note that for $d = 1$ and $d \geq 4$, $n'_d = n_d$ and that $\sum_{d \geq 1} n'_d = l$. Set S has three types of vertices of degree 3: (i) vertices of degree 3 from 2-connected components; (ii) vertices of degree 3 created by edges attached to 2-connected components; and (iii) $n_3 - n'_3$ of vertices of degree 3 which are not part of any 2-connected component. Note that a contracted vertex of degree d in T corresponds to d vertices of degree 3 of type (ii). Therefore,

$$K_3(S) = \sum_{i=1}^{l} K_3(S_i) + \sum_{d \geq 1} d.n'_d + n_3 - n'_3 = \sum_{i=1}^{l} K_3(S_i) + 2l + \sum_{d} (d-2)n_d \,.$$

It can be easily shown by induction that for any tree, $\sum_d (d-2)n_d = -2$. We know that the lemma holds for every 2-connected component, i.e., for every $i = 1, \ldots, l$, $K_3(S_i) \leq 2K_{\hexagon}(S_i) - 2$. Plugging these two facts into formula for K_3 we obtain

$$K_3(S) \leq 2 \sum_{i=1}^{l} K_{\hexagon}(S_i) - 2l + 2l - 2 = 2K_{\hexagon}(S) - 2 \,.$$

Finally, by summing the bound for each connected component of S, we obtain the desired bound for any S. □

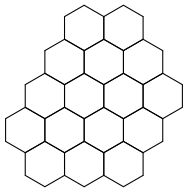


Figure 12: Example of a deformed hexagonal shape with sides 3,3,3,2,4,2.

Lemma 7. *Let S be any set of vertices in a hexagonal plane such that each vertex of S has a degree at least 2. We have $K_{\hexagon}(S) \leq \frac{1}{12}(K_2(S)^2 + K_2(S) - 30)$.*[1]

[1]Note that this is not a tight bound. We conjecture that the following bound holds $K_{\hexagon}(S) \leq \frac{1}{12}(K_2(S)^2 - 6K_2(S) + 12)$.

Proof. First, assume that S is complete and 2-connected, and that its boundary does not have two consecutive concave angles, i.e., the boundary forms a deformed hexagonal shape, cf. Figure 12. We will show that lemma holds for any such deformed hexagonal shape by induction on $K_2(S)$, which is now equal to the sum of its six sides (measured in the number of hexagons on the particular side). It is easy to verify that the lemma holds in the base case when there are two neighboring sides equal to one. Indeed, in this case hexagonal shape is formed by a linear chain of t hexagons and the number of vertices of degree 2 is $2t + 4$. Assume it is not a base case and let s be the shortest side of the hexagonal shape S. Observe that the neighboring sides to s are longer than 1. Consider a hexagonal shape S' obtained from S by removing a row of hexagons on the side s. The number of hexagons $K_{\hexagon}(S')$ is $K_{\hexagon}(S) - s$ and since side s was prolonged by 1, while the neighboring sides shortened by 1, $K_2(S') = K_2(S) - 1$. By induction hypothesis, $K_{\hexagon}(S) - s = K_{\hexagon}(S') \leq \frac{1}{12}(K_2(S')(K_2(S') + 1) - 30) = \frac{1}{12}(K_2(S)(K_2(S) - 1) - 30)$. Since, s is the shortest side of S, $K_2(S) \geq 6s$, and hence

$$\begin{aligned} K_{\hexagon}(S) &\leq s + \tfrac{1}{12}(K_2(S)(K_2(S) - 1) - 30) \\ &\leq \tfrac{1}{6}K_2(S) + \tfrac{1}{12}(K_2(S)^2 - K_2(S) - 30) = \tfrac{1}{12}(K_2(S)^2 + K_2(S) - 30)\,. \end{aligned}$$

Second, assume that S is complete and 2-connected. We will transform S to a new set S' by repeating the following process until possible: if there are two or three consecutive concave angles on the boundary add the vertices of the hexagon they are part of, to S. It is easy to see that this process must stop (we will never go outside of any hexagonal shape enclosing S). Note that in each step $K_{\hexagon}$ increases by 1 and K_2 either stays the same or decreases by 1. Thus $K_{\hexagon}(S) \leq K_{\hexagon}(S')$ and $K_2(S') \leq K_2(S)$. Since, S' is a hexagonal shape and complete, the lemma holds for it. Thus it holds for S as well: $K(S) \leq K(S') \leq \frac{1}{12}(K_2(S')^2 + K_2(S') - 30) \leq \frac{1}{12}(K_2(S)^2 + K_2(S) - 30)$.

Third, assume that S is 2-connected, but not complete. Let $\bar{S}$ be the completion of S as in the proof of Lemma 6. Note all vertices of degree 2 in $\bar{S}$ are on the boundary $B(\bar{S}) = B(S)$ and they must be vertices of degree 2 in S as well. Hence, $K_{\hexagon}(S) = K_{\hexagon}(\bar{S})$ and $K_2(S) \geq K_2(\bar{S})$. Since $\bar{S}$ is complete and 2-connected, it satisfies the lemma. It follows that S satisfies the lemma as well.

Finally, we prove that any set S satisfies the lemma by induction on the number of 2-connected components. Let S' be a 2-connected component of S with at most one edge to $S - S'$. Clearly, such a component exists. If S' is not connected to $S - S'$, let $S'' = S - S'$. Otherwise, let $P = (x, \ldots, y)$ be the path such that x is the only vertex of P in S', all inner vertices $I(P)$ of P have degree 2 and y has degree 3. Then let $S'' = S - S' - I(P)$. Note that $K_{\hexagon}(S) = K_{\hexagon}(S') + K_{\hexagon}(S'')$ and $K_2(S) \geq K_2(S') + K_2(S'') - 2$. Furthermore, S'' satisfies the lemma by induction hypothesis and S' as well, since it is a 2-connected set. Easy calculations and the fact that $K_2(S'), K_2(S'') \geq 6$ show that S satisfies the lemma as well. □

Corollary 1. *Let S be any set of vertices in a hexagonal plane such that each vertex of S has a degree at least 2. We have $K_3(S) \leq \frac{1}{6}(K_2(S)^2 + K_2(S) - 30) - 2c$, where c is the number of connected components of S.*

4.5 Limiting certain types of connections and vertices

In this subsection we limit certain types of connections and vertices that occur in a saturated fold F of q. We first prove that there are at most 4 v-vertices in F.

Claim 3. *Let F be a saturated fold of q and assume it contains a complex component C without a* vv*-vertex. Let s be the smallest integer such that C_s is different from C_1 and let e be the largest integer such that C_e is different from C_r. Let w_1 be the length of the path in C_s and w_2 the length of the path in C_e. Then $w_1 + w_2 \leq 40$.*

Proof. First, note that w_1 and w_2 are well-defined, as by Lemma 5, C_s and C_e contain only one path. Let $(p_1, \ldots, p_{w_1})$ be the path in C_s. Obviously, vertices $p_1^{-1}, \ldots, p_{w_1}^{-1}$ are h-vertices. Let p_0^{-1} ($p_{w_1+1}^{-1}$) be the other neighbor of p_1^{-1} ($p_{w_1}^{-1}$). Both, p_0^{-1} and $p_{w_1+1}^{-1}$, are vh-vertices, otherwise we have an occurrence of substring H0HH. Hence, all vertices, $p_0^{-s+1}, p_1^{-s+1}, \ldots, p_{w_1+1}^{-s+1}$, are vh-vertices. Therefore, in layers C_1 and C_s we have at least $w_1 + 4$ S-vertices. Similarly, in layers C_e and C_r we have at least $w_2 + 4$ S-vertices. Hence, by Observation 7, C contains at least $w_1 + w_2 + 12$ S-vertices. Since, q contains 52 S-vertices , the claim follows. □

Lemma 8. *Let F be a saturated fold of q. No* v*-vertex can be part of substring* $(00HH)^{356}$*. Consequently, there are at most 4* v*-vertices in F.*

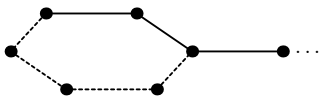


Figure 13: An example of extending the wall's end in layer eliminating vertices with horizontal degree 1.

Proof. Note that since each complex component introduces at least 4 occurrences of H0H, there is at most one complex component in F. Assume to the contrary that the substring $(00HH)^{356}$ contains a v-vertex. By Lemma 1, the substring contains only v-vertices. Let $P_1, \ldots, P_k$ be all hexagonal planes containing these v-vertices and let S_i be the set of H-components in the plane P_i which contain at least one of these v-vertices and let S be the union of $S_1, \ldots, S_k$. Since every component is either a tube, a 2-layer component, a complex component with six vv-vertices, a basic complex component or an appendix complex component, we have the following observations:

- The set S contains only layers of 2-layer components, complex components with vv-vertices, the lower and upper parts of a complex components without vv-vertices if they are 2-layer components and layers of appendix of appendix components. Since all these layers come in identical pairs with exception of vv-component in which 2-layers differ in 6 vertices, we will consider only one layer in the pair. From each pair select only one layer, for the vv-component select the layer with vv-vertices. Let $J \subseteq \{1, \ldots, k\}$ be the set of the selected layers and let $M = \cup_{i \in J} S_i$. We have $K_2(M) \leq K_2(S)$ and $K_3(M) \geq \frac{1}{2} K_3(S)$.
- All vertices have horizontal degree 2 or 3 with exception of the wall and (possibly) appendix of a complex component without vv-vertices. The layer of a wall without appendix contains two vertices with horizontal degree 1, but no vertex with horizontal degree 3, hence, it is not included in M. On the other hand, a layer containing the appendix contains exactly one vertex with horizontal degree 1. Let us extend the path ending in this vertex in its layer until we join another H-vertex, see an example in Figure 13. There is always a way how to do it which introduces at most 4 new vertices with horizontal degree 2, and would always eliminate at least one such vertex. Let M' be the set M extended by these elements and S'_i either S_i or S_i extended by these elements if S_i was the component containing the appendix. Hence, since there is at most one complex component an it contains at most two layers with appendix, we have $K_2(M') \leq K_2(M) + 3$ and $K_3(M') \geq K_3(M)$.

By Corollary 1, we have

$$\begin{aligned} K_3(M) \leq K_3(M') = \sum_{i \in J} K_3(S'_i) &\leq \frac{1}{6} \sum_{i \in J} (K_2(S'_i)^2 + K_2(S'_i)) - 7k \\ &\leq \frac{1}{6}(K_2(M')^2 + K_2(M')) - 7 \leq \frac{1}{6}(K_2(M)^2 + 7K_2(m)) - 5\,. \end{aligned} \tag{1}$$

It remains to upper bound the number of vertices with horizontal degree 2. Such vertices are either vh-vertices or h-vertices. There is at most 52 vh-vertices. If we examine all possible components, we can see that h-vertices are in the inner layers of tubes or in the last (first) layer of the lower (upper) part of the complex components which are directly attached to the walls. However, the component in the inner layer of tube contains only vertices with horizontal degree 2, hence, it does not belong to S. Since, we have at most one complex component, by Claim 3, we have at most 40 h-vertices which are in S. At most half of these vertices are in T, hence, $K_2(M) \leq (52 + 40)/2 = 46$. By (1), we have

$$K_3(S) \leq 2K_3(M) \leq \frac{1}{3}(46^2 + 7 \times 46) - 10 < 711\,.$$

Since, every v-vertex has horizontal degree 3, by the assumption, we have $K_3(S) \geq 2 \times 356 = 712$, a contradiction. □

4.5.1 (S\h)-connections

Corollary 2. *Let F be a saturated fold of q. Then F contains* 36 (S\h)*-connections.*

Proof. By Observation 2, F contains 36 (S\D)-connections. Each D-vertex in such a connection is part of the substring $(\mathsf{00HH})^{356}$, hence, by Lemma 8, is an h-vertex. □

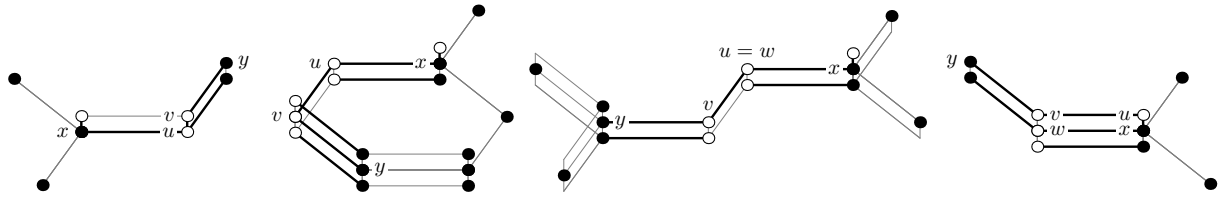


Figure 14: (a-c) Illustration of an external horizontal (S\h)-connection. Contradictory cases: (a) the case when $v = u^1$, (b) the case where x and y are on the same hexagon. The only possible configuration in (c). (d) Illustration of a vertical external (S\h)-connection.

Claim 4. *Let F be a saturated fold of q. Let x be a* vh*-vertex and y be an* h*-vertex in two different components W_1 and W_2. Then there are two types of* (S\h)*-connections* horizontal *and* vertical*. In the horizontal* (S\h)*-connection x and y are on the same plane (cf. Figure 14(c)) and in the vertical* (S\h)*-connection x and y are on two consecutive planes (cf. Figure 14(d)). Furthermore, a vertical* (S\h)*-connection creates an* H0H*-connection between x and a vertical neighbor of y. Finally, if W_1 and W_2 are non-complex, there is at most one parallel (*S\h*)-connection with (x, u, v, y) and in the vertical case the two components share only one layer.*

Proof. Let x be on plane H_i. Without loss of generality assume that x^1 is a 0-vertex and let w be the horizontal 0-neighbor of x. Clearly, u is either x^1 or w. We consider each case separately.

Case 1 ($u = w$). If $v = u^1$ then y must be a horizontal neighbor of v and thus, u is adjacent to the H-vertex y^{-1}, a contradiction (cf. Figure 14(a)). Furthermore, if $v = u^{-1}$ then $y = x^{-1}$ and it follows that x and y are in the same component, a contradiction. Therefore, v is a horizontal neighbor of u and y is a horizontal neighbor of v. Note that y must be the horizontal neighbor of v that is not on the same hexagon with x otherwise, x and y would be in the same component, a contradiction, cf. Figure 14(b). Hence, x and y are on the same plane (horizontal (S\h)-connection), cf. Figure 14(c). Next, assume that (x^i, u^i, v^i, y^i) and (x^j, u^j, v^j, y^j) are two parallel (S\h)-connections with (x, u, v, y). Obviously, $i, j < 0$, and let $i < j$. Since (x, u, v, y) and (x^i, u^i, v^i, y^i) are parallel connections, all vertices between x and x^i (y and y^i) are H-vertices, i.e., neither x^j nor y^j is an vh-vertex. If the components they are contained in are non-complex, they must be D-vertices, a contradiction.

Case 2 ($u = x^1$). By a similar argument used in the first case we can show that $v \neq u^1$. Therefore, v is a horizontal neighbor of u. Since y is an h-vertex none of its vertical neighbor can be a 0-vertices hence, $v = w^1$. It follows that y is vertical neighbor of v and it is on plane H_{i+1}, cf. Figure 14(d). This type of (S\h)-connection is called a vertical (S\h)-connection. Furthermore, in this setting (y^{-1}, w, x) form an H0H-connection. Second, note that y^{-1} is an S-vertex. If the component containing y^{-1} is non-complex, then it is a vh-vertex, i.e., y^{-2} is 0-vertex and the two components can share only one layer. Consequently, there is at most one parallel (S\h)-connection to (x, u, v, y). □

4.5.2 H0H-connections

Definition 6. We say that an H0H-connection is horizontal, vertical if both peptide edges of the connection are horizontal, vertical, respectively.

We have the following simple observation.

Observation 10. *Let F be a saturated fold of a sparse protein of length at least 5. Then every* H0H*-connection connecting two different components is either horizontal or vertical.*

Proof. Assume that H0H-connection (x, y, z) is neither horizontal nor vertical. Without loss of generality, assume that the edge (x, y) is vertical, let $y = x^1$, and (y, z) is horizontal. If z^{-1} is a 0-vertex then we have a closed path of length 4. If z^{-1} is an H-vertex then x and y belong to the same component. □

Claim 5. *Let F be a saturated fold of q and let C be a component of F. Assume that C_i is a layer in F that does not contain any vertex of plane degree 1. Then there is no* H0H*-connection with both end points in C_i. Consequently, there is no internal* H0H*-connection in a tube or a 2-layer component.*

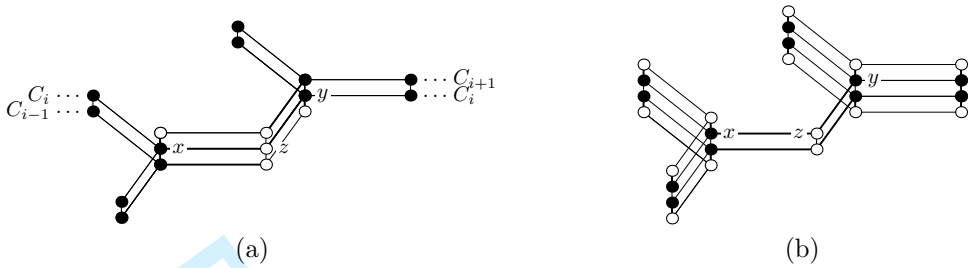


Figure 15: Horizontal H0H-connection (x, z, y): (a) the case where y^{-1} is 0-vertex, (b) the case where y^1 is 0-vertex.

Proof. To the contrary assume that x and y have a common 0-neighbor z. We remark that component C cannot be a vv-component since such a component already contains 6 H0H-connections which are different type than (x, z, y). Clearly one of the vertical neighbors of x has to be a 0-vertex otherwise F contains an occurrence of H0HH as a substring. Without loss of generality assume that x^1 is a 0-vertex. Similarly one of the vertical neighbors of y has to be a 0-vertex. First assume that y^{-1} is a 0-vertex, cf. Figure 15(a). Note that in this case, layers C_{i-1}, C_i and C_{i+1} are different which cannot happen in any component of F. Therefore, x and y are in different components, a contradiction.

Second assume that y^1 is a 0-vertex. It follows that y^{-1} is an H-vertex. Note that x^{-2} and y^{-2} are 0-vertices, otherwise F would contain H0HH as a substring cf. Figure 15(b). Moreover, all horizontal neighbors of y^1, y^{-2}, x^1 and x^{-2}, except z^1 and z^{-1} are 0-vertices, otherwise F would contain an occurrence of the substring H0H0H. Next consider the H0H connection (x, z, y). One of the vertices x and y has to connect to a D-vertex w via two 0-vertices u and v. By Lemma 8, w must be an h-vertex. It is easy to see that $u = x^1$ and $v = x^2$. Now w must be a horizontal neighbor of x^2 which is not possible. □

Corollary 3. *Let F be a saturated fold of q. Then the smallest non-simple tube contains 7 hexagons and 36* S*-vertices, cf. Figure 7(b).*

Lemma 9. *Let F be a saturated fold of q. Consider an* H0H*-connection (x, y, z) connecting two non-complex components W_1 and W_2. If this connection is horizontal then at least one the two components is a tube with more than two layers, they share only one plane and they are configured as in Figure 16(b). If this connection is vertical then they do not share any plane.*

Proof. First, assume that (x, y, z) is a horizontal H0H-connection. It is easy to see that W_1 and W_2 make another horizontal H0H-connection (x', y', z'), cf. Figure 16(a). Note that one of the vertices x or z must connect to a D-vertex w through two 0-vertices u and v. Without loss of generality, let it be x. Obviously, x is a vh-vertex. Without loss of generality, assume that $u = x^1$. By Lemma 8, w must be an h-vertex, therefore, w is a horizontal neighbor of v. Now, if $v = u^1$ then u will be adjacent to the H-vertex w^{-1} (cf. Figure 16(a)), a contradiction. Hence, v is a horizontal neighbor of u and it is easy to see that $v = y^1$ and $w = z^1$. The configuration of parts of two components is depicted in Figure 16(b). Since, the h-vertex w belongs to W_2, W_2 must be a tube with height more than 2 layers and since these two components are non-complex, they can only share one plane.

Second, assume that (x, y, z) is a vertical H0H-connection. Obviously, the two components do not share any plane, and all H0H-connections between them are vertical. An example of configuration in which two non-complex component are vertically H0H-connected is depicted in Figure 16(c). □

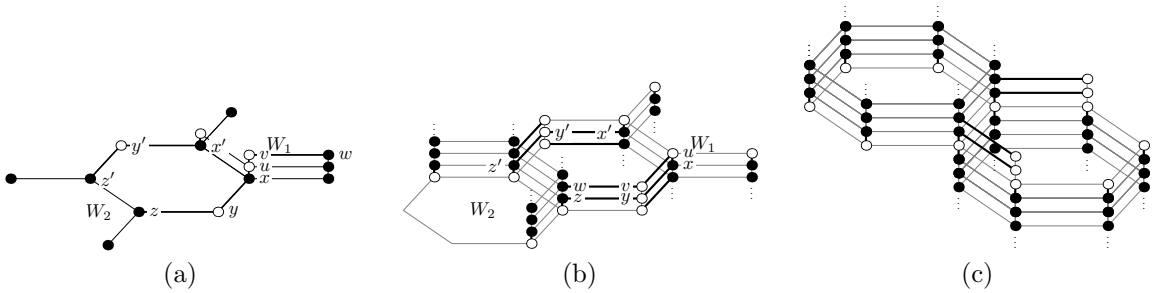


Figure 16: Situation when two non-complex components are connected with a horizontal H0H-connection: (a) x is connected to an h-vertex w away from the other component; (b) w belongs to the other component. (c) An example of two non-complex components connected with a vertical H0H-connection.

4.6 Limiting the possible configurations of complex components

In this subsection we show that only a limited number of configurations are possible for a complex component. This will greatly simplifies our analysis in the later sections. In the following arguments we say that a path has length k if it contains k vertices.

Lemma 10. *Let F be a saturated fold of q. Then F does not contain any* vv*-component.*

Proof. Let C be a vv-component. Consider any of the H0H-paths in C for example (x_1, x_1^1, y_1^1), cf. Figure 8. Notice that this path has to continue with substring $(00HH)^{k_i}$ at one end. By Lemma 8, all H-vertices in this substring are h-vertices, i.e., either y_1^1 or x_1^{-1} has to 00-connect to an h-vertex. It is easy to check that none of these connections is possible, a contradiction. □

Lemma 11. *Let F be a saturated fold of q and let C be a complex component in F with layers $C_1, C_2, \ldots, C_r$. Layer C_1 and similarly C_r is either one hexagon or consists of two hexagons attached by one edge or connected by a path (cf. Figure 18).*

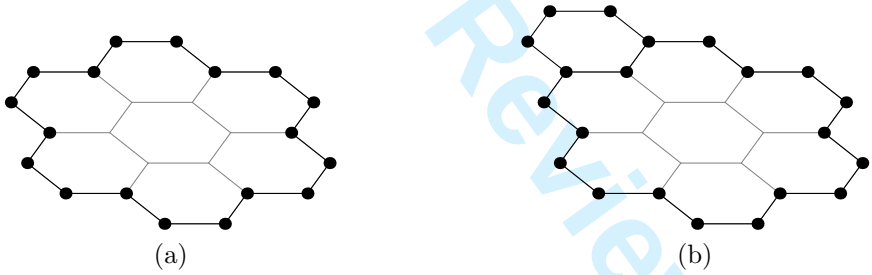


Figure 17: (a) The second smallest cycle without H0H occurrences. (b) The smallest possible layer C_1 of a complex component with the lower part being a 2-layer component containing a large cycle.

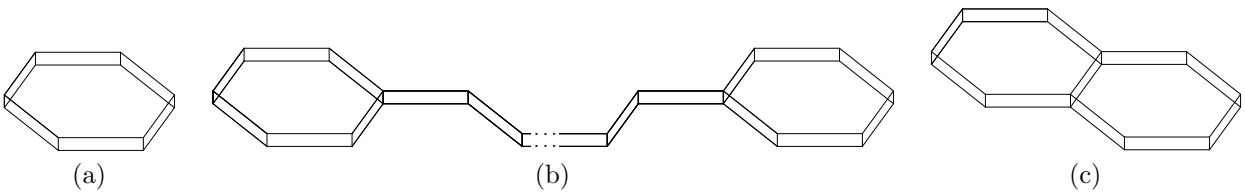


Figure 18: Possible configurations for the upper and lower part of a complex component.

Proof. By Lemma 10, C does not contain any vv-vertex. We prove the claim for C_r. The proof for C_1 follows by symmetry. By Lemma 5, C_r does not contain any horizontal H0H-connection. Furthermore, C_r

cannot contain more than 2 vertices of plane degree 3 because otherwise we get more than 4 v-vertices, a contradiction by Lemma 8. Hence, C_r has one of the three topologies depicted in Figure 18, however, each hexagon could be replaced with larger cycle. We will show that this does not happen.

The smallest possible component layer with no vertex of plane degree 3 other than a simple hexagon is a cycle containing 7 hexagons inside, cf. Figure 17(a) and the smallest possible layer with exactly two vertices of plane degree 3 and at least three hexagons is depicted in Figure 17(b). We prove that C_r cannot be the cycle in Figure 17(a) by computing the lower bound on the number of S-vertices in F. Clearly C will have more S-vertices if C_r has two vertices of degree 3 as in Figure 17(b).

Assume the contrary. We will consider two cases: C is either a basic or an appendix complex component.
Case 1. Let C be a basic complex component. Note that the number of S-vertices in C is minimized when the wall width is maximized and the wall height is minimized. The lower part of C is either a simple tube or the second smallest tube similar to C_r. Figure 19(a)-(b) depicts these configurations with the smallest number of S-vertices. The width of the wall can be at most 4 and 16 in the first and second configurations, respectively. However, in both of these configurations the number of S-vertices is at least 44 which happens when the height of the wall is 2. In addition, notice that C only contains 4 H0H-connections, therefore, F must contain another component which brings the total number of S-vertices up to at least $44 + 12 = 56$, a contradiction.
Case 2. Let C be an appendix component and let w_1 and w_2 be the lower and the upper wall width of C, respectively cf. Figure 19(c). Similar to case 1, the lower part of C is either a simple tube or the second smallest tube. If it is the second smallest tube then the minimum number of S-vertices will be $(18 + 2).2$ (vertices in lower and upper part) + 22 (vertices in appendix and wall ends) = 62, a contradiction. Hence, assume that the C_1 consists of one hexagon. Note that $w_1 \leq 4$ and $w_2 \leq 16$. The minimum number of S-vertices in different layers of C is as follows:

- *vertices in C_r:* 18
- *vertices in the first layer of upper part:* $18 - w_2$
- *vertices on walls ends:* 8
- *vertices of the appendix:* the appendix without the arm contains at least 10 S-vertices, the arm on its ends contain 4 and if the walls have different widths, then on the side of the shorter wall the arm has additional $|w_2 - w_1|$ S-vertices. Hence, in total appendix has at least $14 + |w_2 - w_1|$ S-vertices.
- *vertices of the first layer of the upper part:* $6 - w_1$
- *vertices in C_1:* 6

Hence, the total number of S-vertices is at least $70 - w_1 - w_2 + |w_2 - w_1|$. Now, if $w_1 \leq w_2$ then the minimum number of S-vertices is $70 - w_1 - w_2 + |w_2 - w_1| = 70 - 2w_1 \geq 62$, a contradiction and if $4 \geq w_1 > w_2$ it is $70 - w_1 - w_2 + |w_2 - w_1| = 70 - 2w_2 \geq 62$, also a contradiction. □

Lemma 12. *Let F be a saturated fold of q and let C be a complex component in F. Then the lower and upper part of C are simple tubes.*

Proof. By Lemma 11, the upper part of C is either a simple tube or one of the 2-layer components depicted in Figure 18(b)-(c). To the contrary assume that one of the parts is not a simple tube, say the upper part. First notice that the upper part contains 4 v-vertices therefore, by Lemma 8, C cannot have an appendix, and the lower part must be a simple tube as well. Therefore, C only contains 4 H0H connections, and hence, F must contain at least one other component T. Furthermore, T has to be a simple tube because if it is a 2-layer component, a complex component or a large tube then F would contain more than 4 v-vertices, more than 6 H0H-connections or more than 52 S-vertices, respectively.

Next we consider two cases for the shape of the upper part of C:
Case 1. Assume that the upper part of C is a connector. By Lemma 3, the width of the wall is 2. Now independent of the height of the wall in C the number of D-vertices modulo 6 in F is 2, a contradiction.
Case 2. Assume that the upper part of C consists of two hexagons connected by a path, cf. Figure 18(c). The wall part of C can either attach to one of the hexagons or the path P connecting the two hexagons,

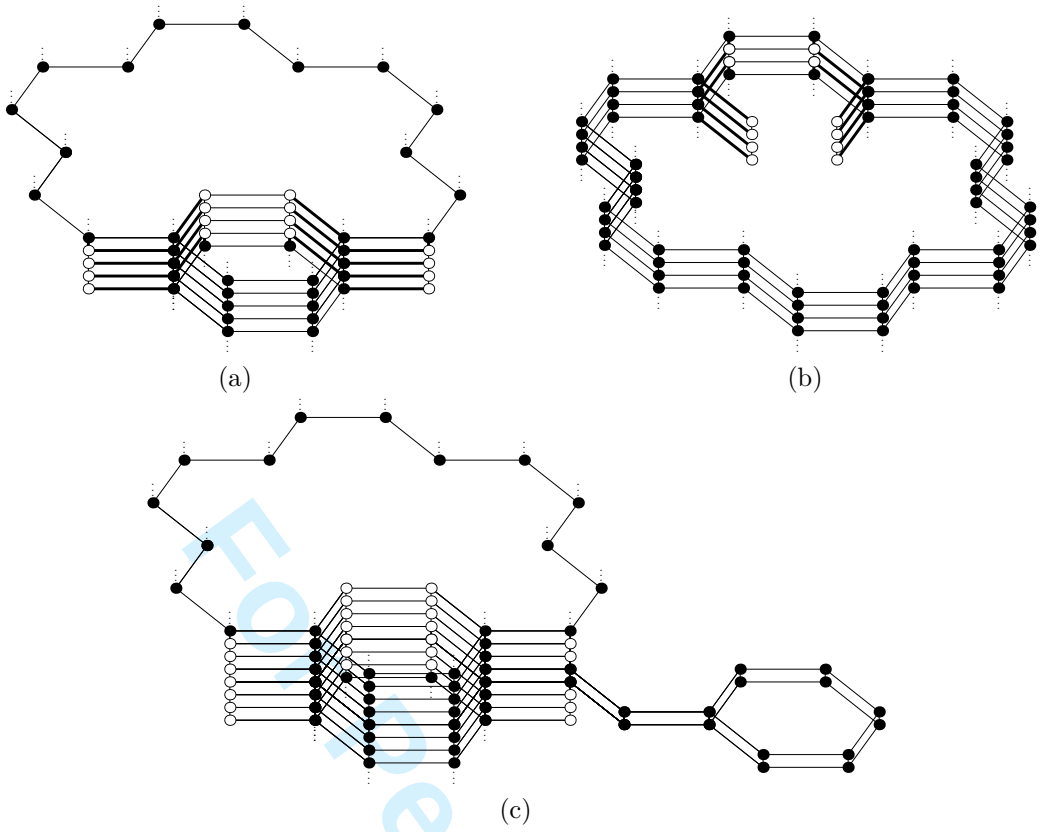
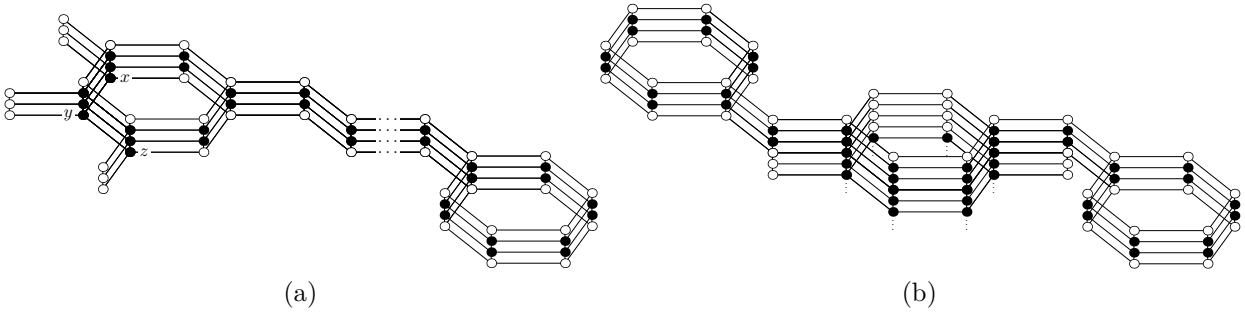


Figure 19: (a) A basic complex component with the second smallest tube as upper part and a simple tube as the lower part. (b) A basic complex component with the second smallest tube as upper and lower part. (c) An appendix component with the second smallest tube as upper part and a simple hexagon as lower part.

cf. Figure 20. Similar to the previous case if the width of the wall is 2 the number of D-vertices modulo 6 in F is 2 independent of height or the location of the wall, a contradiction. Furthermore, if the wall is attached to one of the hexagons then by Lemma 3, the width of the wall can be at most 3. Figure 20(a) depicts this configuration with wall width equal to 3. Let x, y and z be the vertices on the last layer of the wall. Each of the vertices x^1 and z^1 must connect to a D-vertex via a peptide bond. The only D-vertex in their neighborhood is y^1 thus, x^1 and z^1 must both connect to y^1 which is not possible. Using a similar argument we can show that the width of the wall cannot be 3 for the case where it is attached to the path P. Since the lower part is a simple tube the only case remaining for analysis is the configuration in which the wall is attached to P and its width is 4. By Lemma 3, the smallest length of P is 6 and by Observation 7, the smallest height of the wall is 4. Note that such a component would have 40 S-vertices (28 upper part, 4 wall and 8 lower part), and with the extra component at least 52 S-vertices. Increasing either the length of the path or the height of the wall would increase this number hence, Figure 20(b) depicts the only possible configuration of the complex component. We show that this configuration is also contradictory by determining the maximum number of (S\h)-connections possible. Note that at most 12 internal (S\h)-connections are possible across the vertices of C and T, respectively. Therefore, by Corollary 2 we need to create at least 12 external (S\h)-connections between the S-vertices of C and h-vertices of T. However, at least 10 of these (S\h)-connections must be horizontal because each vertical (S\h)-connection create an H0H-connection, by Claim 4. Since a horizontal (S\h)-connection between C and T is possible only when the H-vertices in the connection are on the same plane therefore, C and T must have at least 5 connections per plane which is easy to see it is not possible given the shape of C. □

Lemma 13. *Let F be a saturated fold of q and let C be a complex component in F. The width of the wall in C is either 2 or 4.*

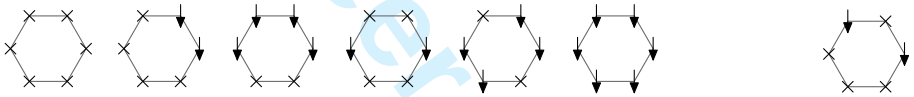


(a) (b)

Figure 20: Examples of complex components with a 2-layer component consists of two hexagons connected by a path as the upper part: (a) wall is attached to one of the hexagons; (b) wall is attached to the path connecting hexagons.

Proof. By Lemma 10, C does not contain any vv-vertex and by Lemma 12, its lower and upper part are simple tubes. Assume that the lower wall starts at layer C_s of C. First observe that the wall width cannot be 1 or 5 otherwise, we get an H-vertex with three 0-neighbors or a 0-vertex with three H-neighbors, respectively, both contradictions.

Therefore, it is enough to show that the wall width cannot be 3. Let x, y, z be the path of the wall in layer C_s (attached to the tube component). Note the number of D-vertices in this layer and above is odd. Since they have to form pairs, y^{-1} has to connect to y, and hence, x and z has to connect to x^1 and z^1, respectively. Let us look at patterns of vertical connections between consecutive layers of a tube. It can be shown by induction (from the top of the tube) that only the patterns depicted in Figure 21(a) are possible. However, the pattern required to realize connections xx^1 and zz^1, depicted in Figure 21(b) cannot be obtained, a contradiction. □



(a) (b)

Figure 21: (a) All possible patterns (up to rotation) for vertical connections between two consecutive layers of a simple tube. The "x" means vertical connection is not present, arrow means it is present. (b) Pattern required to connect to the last layer of a simple tube which is connected to a path of length 3.

4.7 There is no appendix component

Consider an appendix component C in a saturated fold F of q. By Lemma 12, the upper and lower part of C are simple tubes. Let C_a and C_{a+1} be the layers of C that contain the appendix part. Observe that C_a and similarly C_{a+1} contains an odd number of vertices of plane degree 3 (such vertices correspond to v-vertices in C). Therefore, C contains $4k-2$ v-vertices for some positive integer k. By Lemma 8, F contains at most 4 v-vertices hence, the appendix part of C contains one hexagon.

Observation 11. *Let F be a saturated fold of q. Let C be an appendix component in F. Then C contains exactly 2 v-vertices.*

Lemma 14. *Let F be a saturated fold of q. Then F does not contain any appendix component.*

Proof. Assume that F contains an appendix component C. First we show that F can only contain simple tubes. By Observation 9 and Corollary 3, F cannot contain a non-simple tube, otherwise we have too many S-vertices. If F contains another complex component, then we have at least 10 H0H substrings, which is not possible. If it contains a 2-layer component, then F contains at least 6 v-vertices, two in C and 4 in the 2-layer component, a contradiction. Hence, all other components of F are simple tubes. Let N_t be their number.

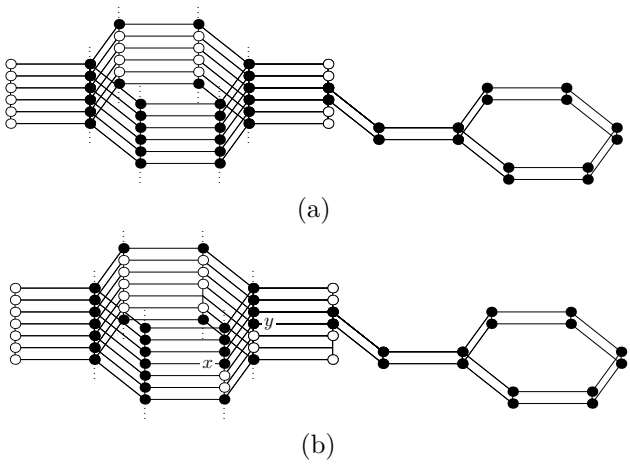


Figure 22: A part of (a) an appendix component with wall of width 4 all along; (b) an appendix component with wall of width 4 and 2 on different sides of appendix.

Let w_1 (h_1) be the width (height) of the lower wall of C and w_2 (h_2) the width (height) the upper wall. Let al be the lengths of the arm. We will calculate the number of D-vertices modulo 6 and the number of S-vertices in C and F. The lower (upper) part of C (a tube) contains w_1 mod 6 (w_2 mod 6) D-vertices modulo 6, the lower (upper) wall $(w_1-2)h_1$ ($(w_2-2)h_2$), the arm of appendix w_1-1+w_2-1 and the remaining part of the appendix, by Observation 11, 2 D-vertices. That is $2(w_1+w_2)+(w_1-2)h_1+(w_2-2)h_2$ mod 6 D-vertices modulo 6 in C, and since all other component are simple tubes, the same number in F. The number of S-vertices is $12-w_1$ ($12-w_2$) in the lower (upper) part, $2h_1$ ($2h_2$) in the lower (upper) wall, $2al-w_1-w_2+2$ in the arm and at least 10 in the remaining part of the appendix. That is at least $36+2(h_1+h_2+al-w_1-w_2)$ S-vertices in C, and $36+2(h_1+h_2+al-w_1-w_2)+12N_t$ in F.

By Lemma 13, both w_1 and w_2 are either 2 or 4, hence, we will consider the following 3 cases (without loss of generality, we assume that $w_1 \leq w_2$).

Case 1. $w_1 = w_2 = 2$. By the above formula, the number of D-vertices modulo 6 in F is 2, a contradiction with Observation 2.

In the remaining two case, we will first show that C is the only component, i.e., that $N_t = 0$.

Case 2. $w_1 = w_2 = 4$, cf. Figure 22(a). By the above formula, the number of D-vertices in F is $4+2(h_1+h_2)$ mod 6. Since, by Observation 2, this number is 4, we have $h_1+h_2 \equiv 0 \mod 3$. Since, by Observation 9, $h_1, h_2 \geq 2$, we have $h_1+h_2 \geq 6$. Also note that $al \geq 5$. Hence, the number of S-vertices is at least $36+2\times 3+12N_t$. Since this number should be 52, we have $N_t = 0$.

Case 3. $w_1 = 2$ and $w_2 = 4$, cf. Figure 22(b). The number of D-vertices modulo 6 in F is $2h_2$ mod 6. Hence, $h_2 \geq 3$. And since $w_2 = 4$, we have again $al \geq 5$. Therefore, the number of S-vertices in F is at least $36+2\times 5+12N_t$. Hence, again $N_t = 0$.

We will determine the maximum number of (S\h)-connections in F. Notice that in any of the configurations the S-vertices in the wall except for the end vertices on the first and the last layers cannot connect to any h-vertex, so there are at most 4 (S\h)-connections involving the S-vertices of the wall components. Furthermore, the S-vertices in the appendix part and its arm can only connect to the h-vertices in the wall that are in the same plane with them otherwise, we get additional H0H-connections, a contradiction. Therefore, there can be at most 4 (S\h)-connections involving the S-vertices of the appendix part and its arm. The last (S\h)-connections that we can get in F are through vh-vertices of the lower and upper tubes, which are 16 in the first configuration and 18 in the second configuration. Two more (S\h)-connections are possible in the second configuration through vh-vertices x and y in Figure 22 (b). Therefore, in total F can contain at most 28 (S\h)-connections, a contradiction, by Corollary 2. □

No other type of possible components can introduce 6 occurrences of H0H, hence, a saturated fold of F contains at least two components. On other hand, since any of possible components has at least 12 S-vertices, we have the following corollary.

Corollary 4. *Any saturated fold of q has at least 2 and at most 4 components.*

In what follows we will analyze all three possibilities. But first, let us have a closer look at tubes.

4.8 Tubes

Lemma 15. *Let F be a saturated fold of q. Any tube in F has either 12 or at least 36* S*-vertices.*

Proof. Obviously, any cycle in a hexagonal plane has at least 6 vertices, i.e., a smallest possible tube will have at least 12 S-vertices. Furthermore, by Claim 5, there is no H0H with both ends in the same tube. The smallest cycle large than a hexagon such that no two non-adjacent vertices are at distance two contains 7 hexagons inside. Thus, the second smallest tube has 36 S-vertices. □

Lemma 16. *Let F be a saturated fold of q. Two* H0H*-connected tubes in F are both simple and furthermore, they make exactly two* H0H*-connections.*

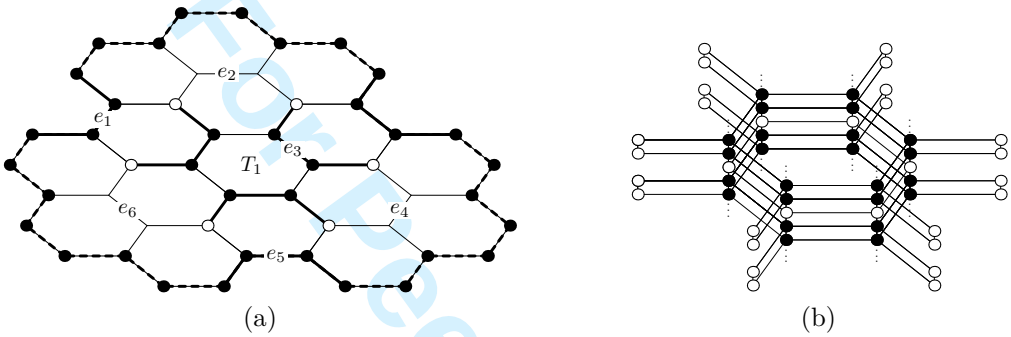


Figure 23: (a) A shortest possible collection of paths connecting the parts of cycle of T_2 that make 6 horizontal H0H-connections with a simple tube T_1. (b) Six vertical H0H-connections between two simple tubes.

Proof. Let T_1 and T_2 be two tubes in F. By Corollary 3, one of them, assume T_1, must be a simple tube. First note that if T_2 is not a simple tube it must make 6 H0H-connections with T_1 since F cannot have another component. Assume that there is an H0H-connection (x, y, z) such that x and z are H-vertices in T_1 and T_2, respectively. By Observation 10, there are two cases:

Horizontal H0H-connection (x, y, z). By Lemma 9, T_1 and T_2 share only one plane H_i and create at least two H0H-connections as depicted in Figure 16(b). We will show that T_2 must also be a simple tube. Assume the contrary. Since T_2 has at least 36 S-vertices, there are no other component in F, and hence, T_2 must make 6 H0H-connections with T_1. Moreover, since T_1 and T_2 share a plane H_i no vertex of T_1 can be above/below any vertex of T_2, i.e, all the H0H-connections are horizontal and they are on plane H_i. Therefore, the only way how to make 6 horizontal H0H-connections is when the large cycle C_2 of T_2 on plane H_i contains 3 parts depicted in Figure 23(a) with thick lines. The shortest collection of 3 disjoint paths which do not create H0H-connection and connecting these parts to one cycle is shown with dashed lines. Note that C_2 would contain at least 30 vertices and hence, T_2 would have more than 60 vh-vertices, a contradiction.

Vertical H0H-connections. Assume that x, y and z are on three consecutive planes H_i, H_{i+1} and H_{i+2}, respectively. In this case, T_1 and T_2 do not share any plane and hence, all the H0H-connections between them must be vertical and in the same planes. Note that if T_2 is not a simple tube it can overlap with T_1 on at most 3 edges creating at most 4 H0H-connections, a contradiction since there are no other components in F. Clearly, two simple tubes could overlap either on 1 or 6 edges creating 2 or 6 H0H-connections, respectively. We show that two simple tubes cannot make 6 vertical H0H-connections. Assume the contrary. Figure 23(b) depicts two H0H-connected simple tubes with 6 H0H-connections. Note that no pair of H0H-connections in this configuration can connect through two 0-vertices. Therefore, F does not contain the substring H0H00H0H which is in q, a contradiction. □

4.9 2 components

Lemma 17. *Let F be a saturated fold of q. Fold F cannot have only 2 components.*

Proof. Assume there are two components in F. Six cases are possible.
Case 1. Assume they are both tubes. By Lemma 16, they can only make two H0H-connections, a contradiction.
Case 2. Assume they are both 2-layer components. By Lemma 8, we have no occurrence of substring $(00HH)^{k_i}$, a contradiction.
Case 3. Assume they are both basic complex components. Then we have 8 occurrences of H0H, a contradiction.
Case 4. Assume one component is a tube T and the other a 2-layer component C. By Lemma 8 and Lemma 15, there are only two configurations with 52 S-vertices. The first configuration consists of a connector and a tube that is the second smallest tube with 7 hexagons inside of its boundary on each layer ($16+36=52$). The second configuration consists of a 2-layer component with two simple tubes connecting by a path of length 11 and a simple tube ($40+12=52$). Note that in both configurations the two components must make 6 H0H-connections. In the first configuration it is easy to see that at most two horizontal H0H-connections can be created between the tube and connector. Therefore, all the H0H-connections must be vertical. However, in this case a v-vertex of the 2-layer component will be part of an H0H-connection creating the substring H0HH, a contradiction. We show that the second configuration is not possible by showing that the maximum number of (S\h)-connections in F is less than 36. Notice that at most two h-vertices of each side of the tube can make (S\h)-connections with vh-vertices of the 2-layer component and hence, we can obtain at most 12 external (S\h)-connections between the 2-layer component and the tube. Considering the 12 internal (S\h)-connections in the tube, the total number of (S\h)-connections in this configuration is at most 24, a contradiction by Corollary 2.
Case 5. Assume one component is a tube and the other a basic complex component. Obviously, the tube must be a simple tube. By Lemma 12, the lower and upper parts of the complex component are both simple tubes. Let w be the width of the wall and h its height. The number of S-vertices is $12+24-2w+2h=52$, so we have $h=w+8$. On the other hand, the number of D-vertices modulo 6 is $2w+(w-2)h$. By Lemma 13, w is either 2 or 4. For, $w=4$ the number of D-vertices modulo 6 is 2, a contradiction. Thus, the only possibility is $w=2$ and $h=10$. Note that if the tube does not connect (through one or two 0-vertices) to an end of the wall then, a substring $(00H)^9$ is created which does not occur in q. Hence, the tube has to connect to both ends of the wall. Figure 24 (a) and (b) depict a birdview at the connection of the wall and a tube (numbered positions) through one and two 0-vertices, respectively. Notice that if the wall connects to tube through two 0-vertices the first two connections have to be horizontal. If the third connection is vertical then we get the configuration in Figure 24(a) in one layer above or below. Clearly, the only way that a tube can be connected to both ends of the wall is when it is in position 2 in Figure 24(a). Notice that in this case tube is connected to both ends of wall through one 0-vertex creating an H0H-connection on each end. Furthermore, there will be at least one parallel H0H-connection on each end and in total at least 4 additional H0H-connection, a contradiction.

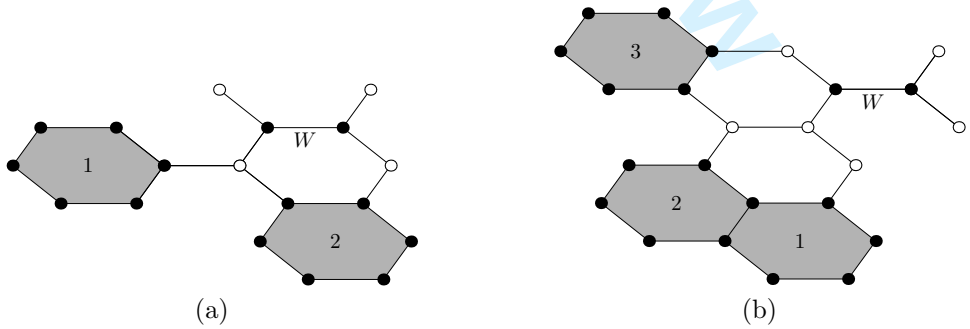


Figure 24: A birdview at the connection of the wall and a tube through one 0-vertex (a) and two 0-vertices (b).

Case 6. Assume one component is a 2-layer component W, and the other a basic complex component C. We show that the maximum number of (S\h)-connections in this configuration is less than 36. First we count

the internal (S\h)-connections in C. All h-vertices in F appear inside the wall and the lower and the upper part of C. The S-vertices of the wall except for the S-vertices on its first and last layers cannot connect to any h-vertex. Therefore, there are at most 4 (S\h)-connections with the S-vertices of the wall. There are at most $12 - w$ S-vertices in the upper (lower) part of C, where w is the wall width. Since $w \geq 2$, there are at most 20 internal (S\h)-connections with the S-vertices of these parts. Therefore, there has to be at least 12 external (S\h)-connections between C and W. It is easy to verify that at most two h-vertices of each side of C can 00-connect to an S-vertex of W. Hence, W has to 00-connect to C from each side. However, one can easily show that for this to happen W must have at least 28 S-vertices in each layer and at least 56 in total, a contradiction.

□

4.10 3 components

Lemma 18. *Let F be a saturated fold of q. Then F cannot contain 3 components where none of them is a complex component.*

Proof. Since, the second smallest tube has 36 S-vertices, all tubes must be simple. Note that F does not contain a complex component and by Lemma 8, F can contain at most one 2-layer component, hence, to obtain 52 S-vertices, F must have two tubes T_1 and T_2, and a 2-layer component W with two hexagons connected by a path of length 5 in each layer.

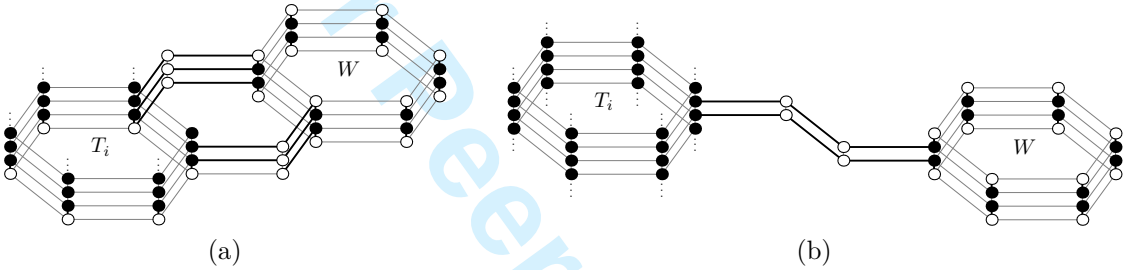


Figure 25: Two possible configurations when a tube T_i and a 2-layer component W are (S\h)-connected: with (a) a vertical (S\h)-connection, (b) a horizontal (S\h)-connection.

By Claim 5, there is no H0H-connection with both ends in W. Therefore, at least one of the tubes, say T_1, must H0H-connect to W. Furthermore, notice that S-vertices of T_1 and T_2 can only provide 24 (S\h)-connections, so we need to create 12 external (S\h)-connections between the S-vertices of W and h-vertices of T_1 and T_2. By Claim 4, these connections are either horizontal or vertical. If W and one of the tubes are vertically (S\h)-connected then we have configuration in Figure 25(a). Notice that although in this configuration two (S\h)-connections are created between the tube and W, we lose two (S\h)-connections across the tube. Therefore, there are 12 horizontal (S\h)-connections between the tubes and W. The only way to create these connections is depicted in Figure 25(b).

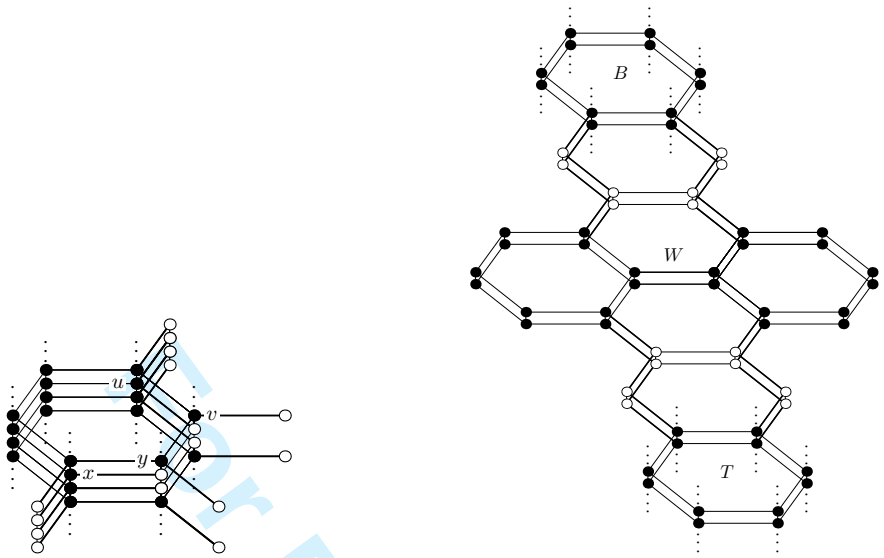
Furthermore, since T_1 and W are H0H-connected, by Lemma 9, none of the h-vertices of T_1 is on the same plane as the vh-vertices of W, and hence, they cannot make any horizontal (S\h)-connections. Therefore, all of the 12 (S\h)-connections must be made between W and T_2. This requires that W connects to T_2 from every side which is not possible since the path connecting two hexagons of W has length only 5. □

Lemma 19. *Let F be a saturated fold of q. Then F cannot contain 3 components where one of them is a complex component.*

Proof. Assume that F contains a basic complex component B. By Lemma 12, B does not have a 2-layer part. Therefore, the number of S-vertices and the number of D-vertices modulo 6 of B are $24 - 2w + 2h$ and $2w + (w-2)h \bmod 6$, respectively where h is the height and w is the width of the wall of B. By Lemma 13, two values are possible for w: $w = 2$ or $w = 4$. We will consider each case separately.

Case 1. **($w = 4$)** Since F contains at most one 2-layer component, one of the three components in F must be a tube T. Furthermore, B has at least 20 S-vertices, therefore, the third component can have at most 20

S-vertices. Hence, it can be either another tube T_2, a connector C or a 2-layer component W that consists of two hexagons connected by one edge in each layer. The values for h are 6, 4 and 2 when the third component is T_2, C or W, respectively. For $h = 6, 4$ the number of D-vertices modulo 6 is 2, a contradiction. Therefore, the only possible configuration is the one in which the third component of F is W and $h = 2$.



(a) (b)

Figure 26: (a) A part of a basic complex component with $h = 2$ and $w = 4$. (b) A configuration with a tube T, a 2-layer component W and a basic complex component B.

The basic complex component B is depicted in Figure 26(a). It has 20 S-vertices, out of which 8 are part of H0H-connections. Notice that only one of the two S-vertices involved in an H0H-connection (such as x and y) can 00-connect to an h-vertex, otherwise F will contain the substring HH00H0H00HH which does not occure in q. Therefore, the maximum number of possible (S\h)-connections with S-vertices vertices of B and T is $16 + 12 = 28$. Hence, we need to create 8 external (S\h)-connections with the S-vertices of W and h-vertices of B or T. Figure 26(b) depicts the only possible configuration to make 8 of such connections. Notice that in this configuration the components are far away to make any H0H-connections with each other so the total number of H0H-connections possible is 4, a contradiction.

Case 2. ($w = 2$) The number of D-vertices modulo 6 of B is 4 independent of the value of h. Therefore, the only possibility for the other two components in F is that they are both simple tubes, say T and T'. To have right number of S-vertices in F the height h must be 4.

Note that an H-vertex from one side of the wall cannot connect to an H-vertex from the other side of the wall through one or two 0-vertices. Therefore, if the wall is not connected to any vertices of T or T' through one or two 0-vertices, then the two H0H-connections on the same side of wall has to connect through a subsequence containing only S-vertices. This creates a substring which does not occur in q, a contradiction. Therefore, at least one vertex on each side of the wall must connect to a tube.

First, we show that the wall cannot 0-connect to a tube. To the contrary assume that a vertex v of tube T is connected to a vertex x of the wall through a 0-vertex w. Vertex x cannot be located on the first or the fourth level of the wall otherwise, F would contain the substring H0H0H, a contradiction. Assume that v is in the hexagon that touches that wall. In this case we get another H0H-connection between other side of the wall and T in the same plane. This situation repeats in the plane above or below. Hence, there are at least 4 new H0H-connections, a contradiction. Now, assume that v is not on the hexagon that touches the wall. The vertex v is a vh-vertex otherwise, F would contain a substring H0HH. Without loss of generality assume v^1 is a 0-vertex. One of the vertices v or x must 00-connect to an h-vertex. It is easy to verify that it cannot be v. Therefore, assume that x connects to an h-vertex of T' through 0-vertices y and y'. The only position of T' is shown in Figure 27. However, in this configuration the right side of the wall cannot connect to neither of the tubes, a contradiction. Therefore, each side of the wall is 00-connected to a vertex

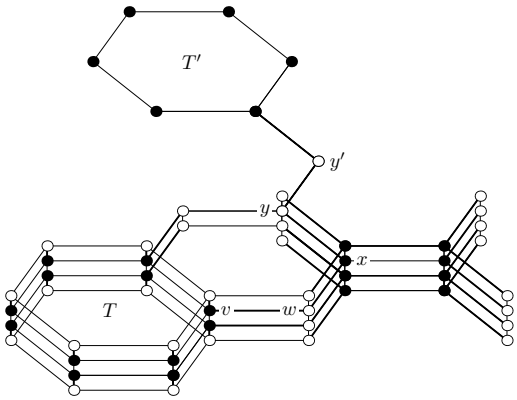
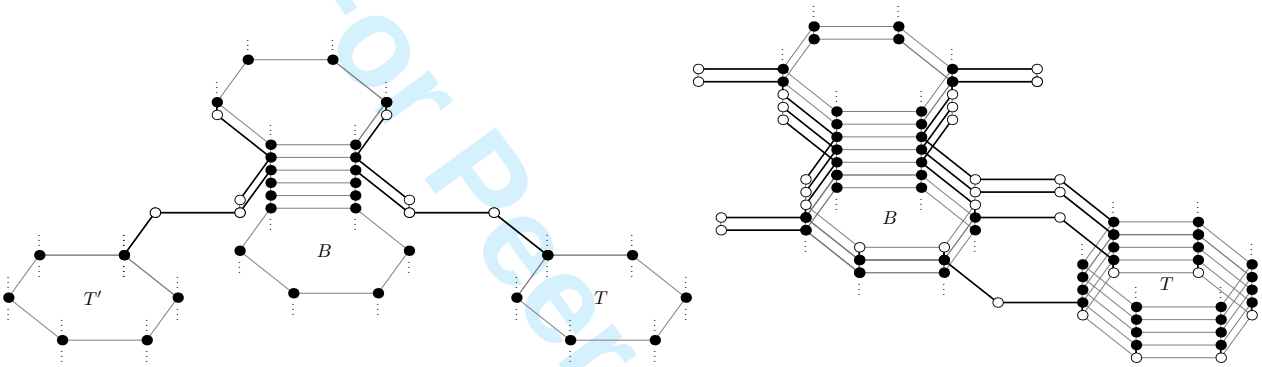


Figure 27: H0H-connections between the tube T and a wall of complex component with $w = 2$ and $h = 4$.

of a tube.



(a) (b)

Figure 28: (a) One possible attachment of two tubes to the wall of complex component. (b) H0H-connections of tube T and basic complex component B.

Notice that it is not possible to 00-connect both sides of the wall to the same tube and hence, one side of the wall is 00-connected to T while the other side is 00-connected to T', e.g., Figure 28(a).

There are two ways to 00-connect a tube to the wall, cf. Figure 29. Note that we need to have two more H0H-connections in F. First, we show that no H0H-connections can be made between B and one of the tubes, say T. Since T cannot H0H-connect to the wall, it would have to connect to the lower or the upper part of B. This is not possible given the relative position of wall of B and T depicted in Figure 29(a). If the relative position of the wall of B and T is as depicted in Figure 29(b), there is only one possible configuration which is depicted in Figure 28(b). However, this configuration contains the substring H0H0H, a contradiction.

Therefore, the H0H-connections must be made between T and T'. The gray hexagons in Figure 29 depicts the possible positions for T'. Clearly, T' cannot 00-connect to the other side of the wall in any of these positions, a contradiction. □

4.11 4 components

So far we have proved that any saturated fold F of q must have exactly four components. In this section we prove that the fold F is similar to the designed fold, i.e., that q is structurally stable. First, we show that the components in F are the same as the components in the designed fold.

Lemma 20. *Let F be a saturated fold of q, then F has three simple tubes and a connector.*

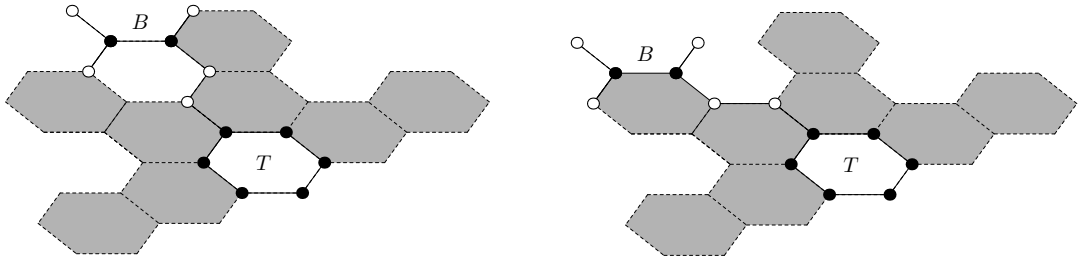


Figure 29: Possible configurations of connecting tube T to the wall of the complex component through two 0-vertices. Gray hexagons represent the locations of T' that can H0H-connect to T and is not too far from the wall.

Proof. Since the smallest component other the tube with one hexagon contains at least 16 S-vertices and F contains exactly four components, F must have three simple tubes and one component other than a tube. The three tubes together have 36 S-vertices, therefore, the forth component in F must have 16 S-vertices. The only component with 16 S-vertices is the connector. Therefore, the components in F are the same as the components in the designed fold. □

Note that the above lemma is true even in the HP model when we do not use properties of cysteines. Next, we prove that in the HPC model the components in F must connect the same way as in the designed fold.

In Lemma 16, two tubes in F can connect with at most two H0H-connections. We will show the same for a tube and connector.

Claim 6. *Let F be a saturated fold of q. A tube and a connector in F can create at most two* H0H*-connections.*

Proof. Assume that the connector C and a tube T are H0H-connected. By Observation 10, this connection is either horizontal or vertical. If the connection is horizontal, by Lemma 9, C and T share only one plane, cf. Figure 16(b). Obviously, all other S-vertices of C and T are too far from each other to create more H0H-connections than the two depicted in the figure.

Second, assume there is a vertical H0H-connection between C and T. Then C and T do not share any plane and H0H-connections are created if an edge of C is directly above/below an edge of T. If C and T overlap on more than one edge, then there is a D-vertex of C directly above/below a vertex of T, which would create a substring H0HH in F, a contradiction. Hence, C and T overlap on only one edge, and hence, create exactly two H0H-connections. □

Claim 7. *Let F be a saturated fold of q. Assume that a connector C and a tube T are horizontally (*S\h*)-connected in F. Then there are at most two external (*S\h*)-connections between them and T is missing at least two internal (*S\h*)-connections.*

Proof. By Claim 4, we have the configuration depicted in Figure 14(d), where y must belong to the tube T and x to the connector C. Vertex x^{-1} is an S-vertex of C and it cannot be part of a parallel (S\h)-connection, because y^{-1} is an S-vertex as well. Also note that S-vertex y^{-1} of T cannot be part of internal (S\h)-connection. Since, horizontal neighbors of y^{-1} and x are H-vertices we have another H0H-connection between these two neighbors and we lose another internal (S\h)-connection. Similarly, there is at most one (S\h)-connection between C and T parallel to this H0H-connection. Considering the layout of C and T, it is clear that they cannot (S\h)-connect at any other point. Hence, the claim follows. □

Observation 12. *Let F be a saturated fold of q. Assume that two tubes T_1 and T_2 are (*S\h*)-connected. Then the number of missing internal (*S\h*)-connections in T_1 and T_2 minus the number of external (*S\h*)-connections between them is at least zero.*

Claim 8. *Let F be a saturated fold of q. Assume that two tubes T_1 and T_2 are* H0H*-connected. Then the number of missing internal (*S\h*)-connections in T_1 and T_2 minus the number of external (*S\h*)-connections between them is at least two.*

Proof. If T_1 and T_2 are vertically H0H-connected then at most one endpoint of each of two H0H-connections is 00-connected to an h-vertex, since there is no HH00H0H00HH in q. Therefore, we lose at least two internal (S\h)-connections and gain no external (S\h)-connections between T_1 and T_2.

If T_1 and T_2 are horizontally H0H-connected we have the configuration depicted in Figure 16(b). Vertices x, x', z, z' are S-vertices of the tubes which cannot be part of internal (S\h)-connections, hence we lose at least four of them. Furthermore, all possible external (S\h)-connections between T_1 and T_2 are (x, u, v, w), $(z, z^{-1}, y^{-1}, x^{-1})$, (x', x'^1, y'^1, z'^1) and $(z', z'^{-1}, y'^{-1}, x'^{-1})$. However, first two and last two cannot be present at the same time, otherwise we have HH00H0H00HH in q. Hence, there are at most two such connections. □

Lemma 21. *Let F be a saturated fold of q. The tubes in F have more than* 3 *layers.*

Proof. Assume that one of the tubes, say T_1, has two or three layers. We prove this lemma by counting the number of possible (S\h)-connections in F. If T_1 has 2 layers, then it does not contain any internal (S\h)-connections, since it has no h-vertices. If it has 3 layers then it contains 6 h-vertices, but since they are connected to each other with a peptide bond and there are only two occurrence of substring 0H00HH00H0 in q which are occurring in the connector, at most one in each pair can be involved in an (S\h)-connection. Hence, T_1 has at most 3 internal (S\h)-connections. There should be 36 (S\h)-connections in F, and the remaining two tubes have at most 24 internal (S\h)-connections. Hence, F must contain at least 9 external (S\h)-connections. By Claim 7, any external vertical (S\h)-connection eliminates at least one internal (S\h)-connection. Hence, there has to be at least 9 external horizontal (S\h)-connections.

Consider an external horizontal (S\h)-connection (x, u, v, y) connecting components W_1 and W_2, cf. Figure 14(c). By Lemma 16 and Claim 6, any pair of components in F can create at most two H0H-connections, i.e, at least three pairs of components are H0H-connected. Since these pairs cannot be horizontally (S\h)-connected, there are at most three pairs of horizontally (S\h)-connected components. Hence, by Claim 2, there are at most 6 horizontal (S\h)-connections, a contradictions. □

We proceed by proving the following lemma.

Lemma 22. *Let F be a saturated fold of q. Any component in F must be* H0H*-connected to at least one other component.*

Proof. By Lemma 15 and Claim 6, there are at most two H0H-connections between any two components of F. Since F contains 6 H0H-connections it is enough to show that there is no cycle of length 3 of H0H-connected components. Let components W_1,W_2,W_3 form such a cycle. By Lemmas 9 and 16 and Claim 6, two H0H-connected components are either in the configuration depicted in Figure 16(b) or Figure 16(c), i.e., they share exactly one plane or they share no planes and there is one plane in between them. Assume that W_1 is the topmost component in planes $H_i, H_{i+1}, \ldots, H_j$. If both W_2 and W_3 share one plane with W_1 (not share any plane with W_1) then they share at least two layers, i.e., they cannot be H0H-connected. Hence, assume that W_2 shares plane H_i with W_1, i.e., it is located in planes $H_i, H_{i-1}, \ldots$ and W_3 does not share any plane, i.e., it is located in planes $H_{i-2}, H_{i-3}, \ldots$. Then W_2 and W_3 can share zero or one plane only if W_2 has either one or three layers. Obviously, the first case is not possible. In the second case, W_2 must be a tube, but by Lemma 21, it cannot have 3 layers, a contradiction. □

We proceed by proving the following important lemma:

Lemma 23. *Let F be a saturated fold of q. Two tubes in F cannot be* H0H*-connected. Consequently, two tubes cannot be vertically* (S\h)*-connected.*

Proof. To the contrary assume that two tubes are H0H-connected. By Claim 7, we need at least two external (S\h)-connections, and by Claim 7 and Observation 12, there are at most two horizontal (S\h)-connections between the connector C and a tube T_1.

Figure 30 shows a birdview at the horizontal (S\h)-connections between T_1 and C. Notice that T_1 and C cannot be H0H-connected. Therefore, by Lemma 22, T_1 must H0H-connect to another tube T_2. We will show that T_2 cannot be H0H-connected to C. Assume the contrary. The tube T_2 must be located in one of the three numbered positions in Figure 30.

Figure 31 depicts configurations for all three positions of T_2. Clearly, in the first configuration T_2 cannot make any H0H-connections with C (cf. Figure 31(a)). Consider vertex v in Figure 31(b) depicting the second

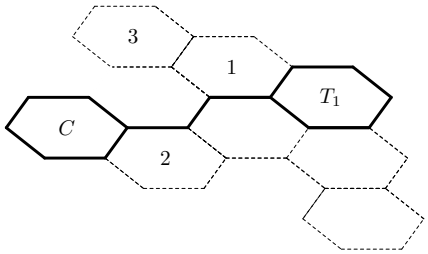


Figure 30: The birdview at horizontally (S\h)-connected connector C and tube T_1. The numbers show all possible locations of tube T_2 which is H0H-connected to both T_1 and C.

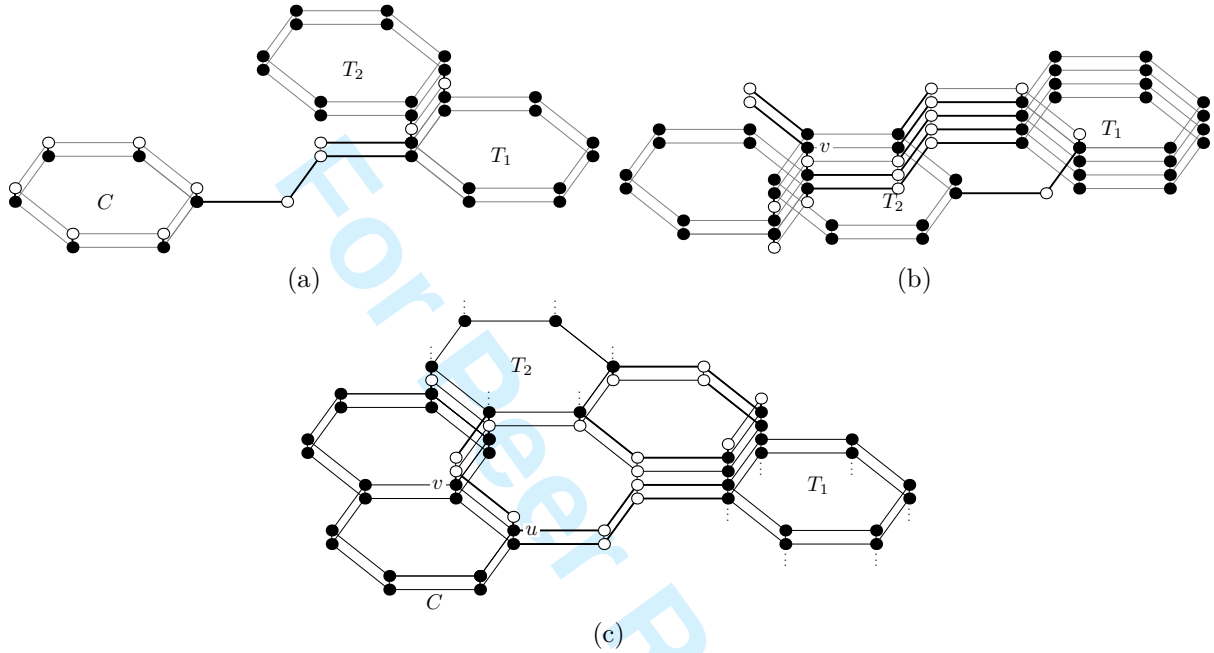


Figure 31: Three possible configurations when connector C is horizontally (S\h)-connected to T_1, and T_2 is H0H-connected to both C and T_1.

configuration. It is H0H-connected to v^{-2} which is part of an (S\h)-connection. Since there is no substring HH00H0H00HH in q, v cannot be part of any (S\h)-connection. Therefore, we lose one more internal (S\h)-connection in T_2 which needs to be replaced by an external (S\h)-connection between C and a tube. By Claim 7, any external vertical (S\h)-connection eliminates at least one internal (S\h)-connection, therefore, the replaced connection must be a horizontal (S\h)-connection. Clearly, T_2 cannot make any horizontal (S\h)-connections with C and furthermore, T_1 cannot make any new horizontal (S\h)-connections with C. Hence, T_3 must make at least one horizontal (S\h)-connection which in this case cannot be H0H-connected to C. Therefore, T_3 must H0H-connect to T_1 or T_2. In this case we lose at least two additional internal (S\h)-connections which cannot be replaced by any external horizontal (S\h)-connections. Finally, we show that the third configuration is contradictory. Consider the v-vertex v in Figure 31(c). If it is 00-connected to w or x it follows that v is a part of the substring $(00HH)^k$, a contradiction by Lemma 8. Therefore, v is 00-connected to u. However in this case, F contains the substring HH00H00HH which does not occur in q, a contradiction.

It follows that T_2 and C are not H0H-connected. Therefore, by Lemma 22, T_3 must H0H-connect to C and to have 6 H0H-connections in F, T_3 must also H0H-connect to T_1 or T_2. However, in this case we lose at least two additional internal (S\h)-connections which by Claim 7, must be replaced by horizontal (S\h)-connections between C and a tube. Clearly, T_3 cannot make such connections with C. Furthermore, T_1 cannot make new horizontal (S\h)-connections with C. Thus, T_2 must make two horizontal (S\h)-connections

with C. Let H_i and H_{i+1} be the layers of C. Without loss of generality assume that T_2 is above T_1. Since C and T_1 make horizontal (S\h)-connections the top most layer H_j of T_1 is above H_{i+1}. Let H_l be be the lowest layer of T_2. Since T_1 and T_2 are H0H-connected, $l \geq j > i+1$. Therefore, C and T_2 do not share any layer and hence, cannot be (S\h)-connected, a contradiction. □

Corollary 5. *Let F be a saturated fold of q. All tubes in F must be* H0H*-connected to the connector.*

Proof. We consider three cases. If the connection is between two h-vertices then clearly all edges of the connection must be horizontal. Second the case when the connection is between h- and S-vertices follows by Lemma 23. Finally, if the connection is between two S-vertices, we lose two internal (S\h)-connections which can be only replaced by horizontal (S\h)-connection between connector and a tube. By Corollary 5, this is not possible. □

So far we have shown that all tubes must H0H-connect to C. We prove the final theorem.

Theorem 1. *The protein string q is structurally stable.*

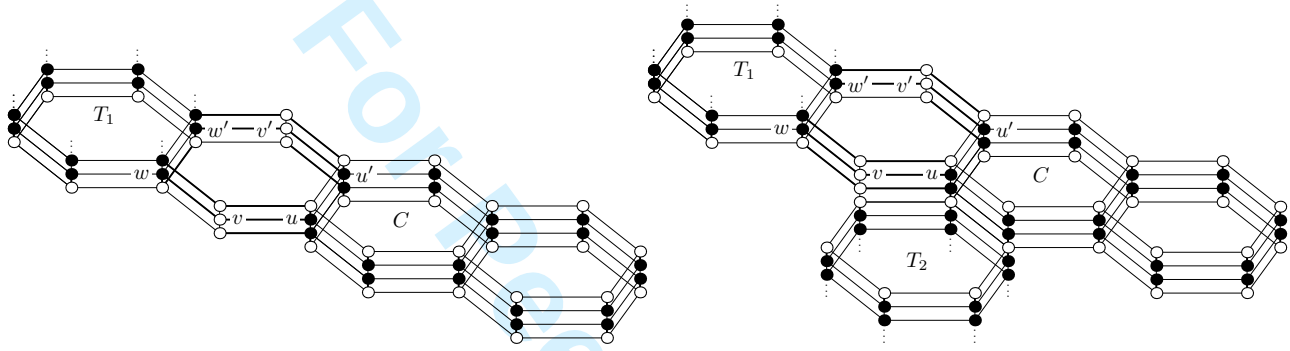


Figure 32: Two possible configurations that contain the substring t = 10100102002, given that one of the H0H-connections in t is horizontal.

Proof. Let F be a saturated fold of q. By Lemma 20 and Corollary 5, F contains three simple tubes which are H0H-connected to a connector C. Note that there are no H0H-connections between tubes. First we analyze the configurations that contain the substring t = 10100102002. The substring t contains two H0H-connections that are 00-connected. We show that these H0H-connections are vertical and they belong to two tubes T_1 and T_2 where T_1 is connected to the top and T_2 is connected to the bottom of C. To the contrary, assume that one of the H0H-connections (u, v, w) in t is horizontal, where u and w are H-vertices in C and T_1, respectively, and v is a 0-vertex. Note that C and T_1 make another H0H-connection (u', v', w') where u' and v' are horizontal neighbors of u and v respectively. Vertex u or w (respectively, u' or w') must 00-connect to an h-vertex. It is easy to see that w (w') cannot 00-connect to an h-vertex and the only h-vertex that u (u') can 00-connect to is w^1 (w'^1). Therefore, w must 00-connect to an H0H-connection.

Two configurations are possible in this case. In the first configuration w is 00-connected to w', cf. Figure 32(a), and hence, exactly one of the pairs of vertices (u, w^1) or (u', w'^1) contains 2-vertices. Since T_1 makes H0H-connections only with C and every 2-vertex is either a part of H0H-connection or is 00-connected to an H0H-connection, w^1 (respectively, w'^1) cannot be paired with a 2-vertex, a contradiction. In the second configuration w is 00-connected to u^{-1} and C is vertically H0H-connected to another tube T_2 at u^{-1} and its horizontal neighbor (cf. Figure 32(b)). Note that T_3 must connect to the hexagon of C the does not contain u and u^{-1} otherwise, F would contain the substring $(00H)^6$, a contradiction. Therefore, T_1 is too far from T_2 and T_3 to 00-connect to either of them. Hence, w^1 is p-connected to w'^1 by a path p which lies completely in T_1 and its 0-vertices (0-vertices surrounding T_1). Consequently, p does not contain any H0H as a substring. Since H0H-connection (w, v, u) is 00-connected to H0H-connection (u^{-1}, u^{-2}, u^{-3}), based on the properties of q, it follows that exactly one of the pairs (u, w^1) or (u', w'^1) contains 2-vertices, depending on the direction of the substring t. Clearly, w^1 (respectively, w'^1) cannot be paired with any other 2-vertex, a contradiction. Therefore, both H0H-connections in t are vertical.

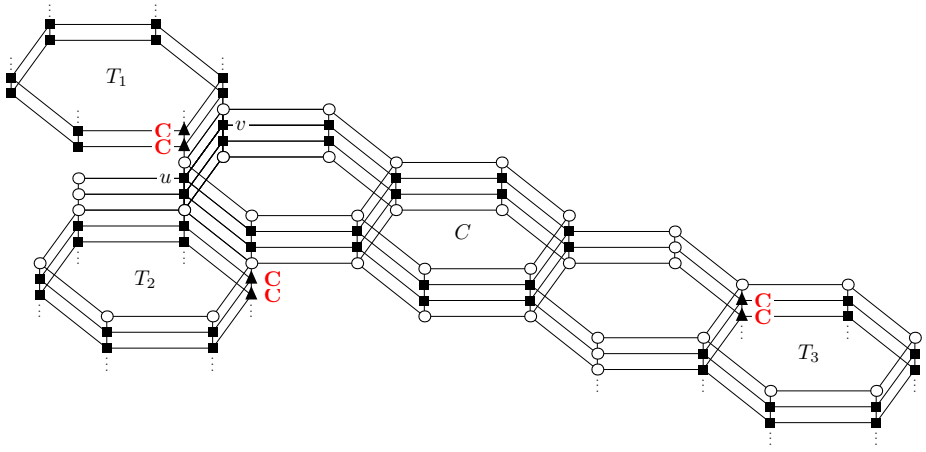


Figure 33: The only possible configuration that contains the substring t = 10100102002, given that the H0H-connections in t are vertical.

Let (u, u^1, u^2) be one of the H0H-connections in t where u and u^2 are H-vertices in C and T_1, respectively, and u^1 is a 0-vertex. Without loss of generality assume that T_1 is connected to the top of C. Note that T_1 and C make another vertical H0H-connection (v, v^1, v^2), where v is a horizontal neighbor of u. Clearly, u cannot 00-connect to an h-vertex, therefore, it must 00-connect to another vertical H0H-connections. The only possibility is that u^1 is 00-connected to u^{-1}. Therefore, C vertically H0H-connect to another tube T_2 at the vertex u^{-1} and one of its horizontal neighbor. If this connection is (v^{-1}, v^{-2}, v^{-3}) then F would contain another occurrence of the substring t through vertices $v^2, v^1, v, *, *, v^{-1}, v^{-2}, v^{-3}$, a contradiction. It follows that T_1 and T_2 are H0H-connected to C as in the original fold. It is easy to see that the last tube T_3 must horizontally H0H-connect to the other side of C as in the original fold (cf. Figure 33).

Finally, notice that T_1, T_2 and T_3 are far away from each other to make any 00-connections. Therefore, the pair of H0H-connections in each tube are p-connected by a path p that lies completely in that tube and its 0-vertices. This implies that the length of the tubes must be the same as the length of the tubes in the original fold and hence, q is structurally stable. □

5 Conclusions

In this paper building on the work done in Gupta et al. (2005, 2007); Hadj Khodabakhshi et al. (2008) we solve the shape-approximating inverse protein folding problem under the HP model in 3D for designing tubular proteins by providing two basic building blocks: a tube and a connector, which can be interconnected to roughly approximate any given shape. We showed that a simple subclass of the structures built in this way is structurally stable in the HPC model. Showing that all these structures are structurally stable is a very challenging problem. The first task in solving this problem is to choose which of the hydrophobic monomers are cysteines. The second is to prove that all folds are similar to the designed one. This gets more difficult with the higher number of building blocks (tubes and connectors) used, as each additional building block adds two special substrings to the protein sequence, and thus increases a variety and the number of possible components in the fold.

While the techniques presented here will not allow for the direct construction of proteins, they represent a starting point for this process. In particular, we believe that our techniques can be used to form the basis of an actual protein — we specify, at each point of the chain whether a cysteine, other hydrophobic or polar monomer is required and a designer can use this information to choose amino acids from set of all 20 amino acids. The choice of actual amino acid would depend on other desired molecular interactions and finer details about the protein structure.

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