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Blueprints for dodecahedral DNA cages

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Abstract

Cage structures engineered from nucleic acids are of interest in nanotechnology, for example as a means of drug delivery (Destito *et al* 2007). Until now, most experimentally realized DNA cages have crystallographic symmetry, such as the shape of a cube (Chen and Seeman 1991 *Nature* **350** 631–3), a tetrahedron (Goodman *et al* 2005 *Science* **310** 1661–5), an octahedron (Shih *et al* 2004 *Nature* **427** 618–21) or a truncated octahedron (Zhang and Seeman 1994 *J. Am. Chem. Soc.* **116** 1661–9). Two examples of cages with non-crystallographic symmetry, a dodecahedron and a buckyball, have been realized recently (He *et al* 2008 *Nature* **452** 198–201). A characteristic feature of these realizations is the fact that the cages are built from a number of identical building blocks called tiles: 20 for the case of the dodecahedron, and 60 for the case of the buckyball. We derive here a blueprint for the organization of nucleic acid in a dodecahedral cage such that the final product has a minimal number of strands. In particular, we show that a dodecahedral cage can be realized in terms of only two circular DNA molecules. We focus on the dodecahedral cage, because the volume to surface ratio of such a cage is larger than that of its crystallographic counterparts given the same fixed radial distance of the polyhedral vertices from the centre of the structure, whilst still requiring a smaller complexity than the truncated icosahedron (buckyball). We therefore expect that the dodecahedral DNA cages discussed here may be of interest in further applications in nanotechnology.

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(Some figures in this article are in colour only in the electronic version)

1. Introduction

Nanotechnology applications make use of structures inspired by nature to perform certain tasks at the nanoscale. For example, nanodevices formed from nucleic acid are used for biomedical

applications [6, 7], and cage structures engineered from DNA are of interest as containers for cargo delivery or storage [1]. DNA cages in the shape of a cube have been constructed [2], and more recently a tetrahedron [3], an octahedron [4] and a truncated octahedron [5] have been obtained. Two cages with non-crystallographic symmetry have been engineered recently [8], and it has been argued in [9] that dodecahedral cages should in principle be realizable from DNA. In both references, the dodecahedral cages are constructed in terms of a number of DNA molecules. It is the purpose of this paper to provide an alternative blueprint for the putative organization of a dodecahedral cage in terms of a minimal number of circular DNA molecules, and we show that the minimal number achievable with our construction method is two. There may be advantages in constructing such cages from a limited number of single-stranded molecules. For example, a single strand could be easily amplified as a PCR product, or cloned, see for example [4]. We expect that such a model will be of interest in nanotechnology applications, because the volume to surface ratio of such a cage is larger for this type of cage, allowing for transportation or storage of a larger cargo at the same overall size of the particle.

The inspiration for this work comes from a theoretical model for the structural organization of the RNA in a dodecahedral cage that has been suggested in [11] in the context of virology. There it is proposed that the double-stranded RNA molecule that encodes the viral genomic material and forms the dodecahedral cage follows a directed path on a dodecahedral graph that visits each edge precisely twice in the opposite direction according to the data in [12] with the two strands oriented in the standard way, one in the $5'$–$3'$ direction and the other in the $3'$–$5'$ direction. The authors in [11] propose a set of vertex configurations that describe potential ways of hybridizing the RNA strands in three-branched junctions corresponding to the three-degree vertices of the dodecahedral cage. Here, we consider DNA instead of RNA cages because synthetic DNA is more stable and often used for assembling nanostructures [21–23]. We consider all possible strand connections at a 3-armed junction vertex. This includes an additional type of connection to those considered by [11], which has been used in the construction of DNA graph structures [13]. We use this extended set of vertex configurations, together with some results on the number of strands in the assembly of thickened graphs in [20], to derive a model for the DNA organization in a dodecahedral cage.

In section 2 we discuss possible ways for hybridization of the strands in three-way junctions as potential realizations of the three-degree vertices of the dodecahedral cage. Each local choice of a connection at a three-way junction enforces a certain configuration of the strands in the global cage structure, and we describe how a local change of connections at a vertex induces a change in the overall strand configuration within the cage. Based on these arguments, we consider two separate cases in section 3: dodecahedral cages whose edges consist of duplex DNA with odd numbers of helical half-turns, and those with an even number of half-turns. Due to the orientation of the strands, in the case of cages with an odd number of half-turns appearance of dsDNA on the edges induces certain strand connections at the vertices. We show that the minimal number of such connections for a dodecahedral cage is six, and that there are five inequivalent ways to obtain the dodecahedral structure. We moreover show that one of these cases is realized by two DNA strands, two by four strands and two by six strands. In the cases of the four-strand and the six-strand solutions, if one (respectively, two) vertices are substituted with the new configuration, the overall number of strands reduces to two. Similarly, in the case of DNA cages with an even number of half-turns per edge, we show that by changing the structures of five vertices, we can obtain a dodecahedral structure with two single DNA strands. In all cases, the structure of the whole cage can be obtained as a single DNA molecule by allowing hairpins at one of the edges that could potentially be joined by a short sticker strand [10].

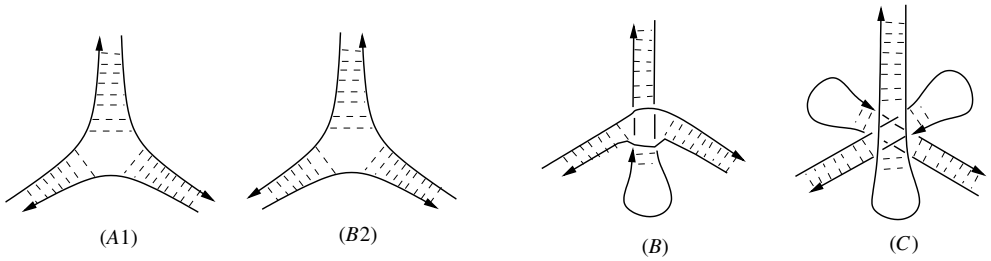

Figure 1. The vertex configurations suggested in [11]: branch point (A1&A2), bubble/hairpin (B) and triple hairpin (C).

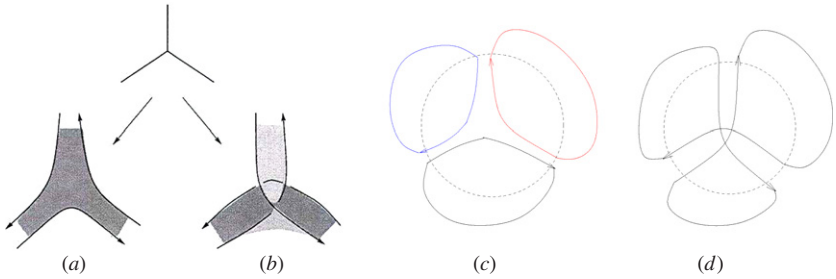

Figure 2. The branch point with a twist (*b*) shown in comparison with a branch point of type (A1) or (A2) in (*a*). Figure adapted from [20]. (*c*) and (*d*), referring to lemma 2.1, show the effect of replacing a branch point of type (A1) or (A2) by the branch point with a twist. The three separate strands are united into one single strand after a replacement of the branch point.

2. Possible vertex configurations

Our starting point is the vertex configurations, i.e., the possible DNA strand hybridizations representing a trivalent vertex. Shown in figure 1 are configurations adapted from [11], called branch point (A1 and A2), bubble/hairpin (B) and triple hairpin (C), respectively, following the terminology introduced in this reference.

The authors of [11] were interested in understanding the RNA cage structure of the genomic material of a virus. Since the genomic material is packaged within a protein shell, configurations B and C may appear. However, if interactions with the capsid proteins are not present and the dodecahedral DNA cage is based solely on the nucleic acids, such vertex configurations cannot sustain the desired structure and only vertices of type A are relevant in this context.

Studies of junction molecules and DNA self-assembly have shown that there is a further possible organization of the strand connections at the vertices [13]. This configuration is illustrated schematically in figure 2(*b*) shown in comparison with the vertex configuration of type A in figure 2(*a*). If the edges of the cage are rigid, meaning, they form a full duplex DNA portion with a base pairing up to the junction, then the different orientations in the two junction types result from a difference of only a few base pairs in the lengths of the DNA strands. If base pairing does not occur close to the junctions the single-stranded portions of the DNA allow flexibility for the strands to turn and the connections to change into the configuration shown in figure 2(*b*). Such flexibility at the junctions was obtained by the introduction of

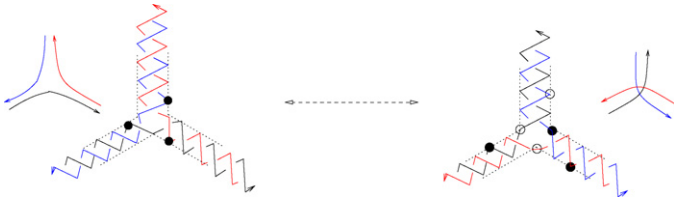

Figure 3. Illustration of the difference in the double-helical structure between branch point, vertex type A (left) and the new branch point with a twist (right). Differences in the length of the DNA sequence of only 3 to 5 nucleotides at one of the edges, as indicated by dots, move to locations shown on the right as filled or unfilled circles. This leads to different orientations of the DNA strands. If the change follows the unfilled circles, then it can be considered as if it removes a twist (half-turn) in the left and right arms, but it adds a twist to the vertical arm. On the other side, if the change follows the bolded dots, then it can be considered as if a twist was added to the left and right arms, but removed from the vertical arm. In both cases, the net change in the number of twists is -1 (former) or $+1$ (latter), and both can be obtained as a small shift of the strands along adjacent vertices.

bulges made of only 2 to 4 unpaired thymines in the construction of the graph in [13]. On the other hand, if there is a shift in length of 3 to 5 nucleotides (figure 3), the new junction type in figure 2(*b*) may be used, which implies another plausible construction scenario. Therefore the set of vertex types that can be used in the dodecahedral folding of the DNA can be extended by this additional configuration which we call *branch point with a twist*.

We note that synthetic DNA is very versatile and can be used to realize a variety of structures, ranging from polyhedra, two-dimensional arrays made with numerous motifs and arbitrary graph structures [14]. In particular, the 3-arm junction types suggested here as building blocks are indeed realizable with synthetic DNA and have been used successfully for the formation of a variety of structures [15, 16]. Non-palindromic sequences also form 5-, and 6-way junctions [17], and recently Seeman's lab reported 8- and 12-arm junctions [18]. Moreover, an approach combining self-assembly and on-surface synthesis may allow formation of unique structures that are not obtainable through self-assembly from conventionally deposited building blocks [19].

We call an arrangement of DNA strands into double-stranded dodecahedral configuration *optimal* if it contains only two strands of DNA. In the next section we determine the overall distribution of vertex configurations in the dodecahedral cage for the optimal scenario.

We will use the following lemma from [20].

Lemma 2.1. *Suppose the strands hybridized in a branched junction of type A (figure 2(a)) are part of three distinct cyclic single-stranded molecules (figure 2(c)) Then after replacing such a junction of type A by a branch point with a twist (figure 2(b)) the strands composing the new junction belong to a single cyclic molecule (figure 2(d)).*

3. Cage assembly

As in [11], we assume that all edges of the dodecahedron are occupied by rigid duplex DNA segments. If we consider the duplex DNA as a ribbon, then due to the helical nature of the duplex structure, the edge lengths of the dodecahedral cage, i.e., the number of helical turns, determine the number of twists that occur in the ribbon along each edge. We start the analysis by assuming that the cage is close to being a Platonic solid, so that the edge lengths are uniform; hence the number of base pairs along the edges of the corresponding DNA cage is

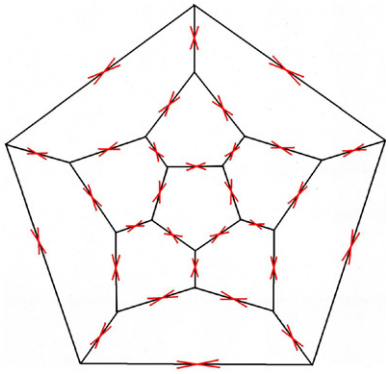

Figure 4. A dodecahedral cage in planar projection, with cross-over schematically representing the twists that occur along the edges as a result of an odd number of half-turns in the helical structure.

more or less constant. We therefore can start by assuming that there are either an even number of half-turns or an odd number of half-turns on each edge. We consider both, the case of an odd, and the case of an even number of half-turn per edge in detail.

3.1. Odd number of half-tuns per edge

We concentrate first on the case where the edge length of the dodecahedral cage is such that the DNA duplex has an odd number of half-turns along each edge, and we start our considerations with a dodecahedral cage in planar projection. The occurrence of the additional twist produced by the odd number of half-turns from the helical structure along each edge is indicated schematically by a red cross-over (see figure 4).

Considering the double helical structure as a ribbon strip whose boundary edges are the DNA strands and placing such a strip along the edges of a pentagonal face, each helical half-turn results in one twist of the ribbon. The resulting number of twists along a pentagonal face is an odd number, and hence the surface represented by the ribbon is non-orientable. As the DNA strands hybridize in an opposite orientation (one strand in orientation $3'$–$5'$ hybridizes with a complementary strand oriented $5'$–$3'$) when forming a duplex, the embedding of the ribbon must correspond to an orientable surface (see, for example [24]). This means that additional half-turns, or removal of half-turns, must appear in the configuration. Figure 3 shows that using a branch point with a twist instead of a branch point results in a net ± 1 twist along an incident edge. Due to the symmetry of the junction, this addition (or removal) of a twist can appear along any of the three adjacent edges of a junction. Figure 5(*a*) shows strand connections in a branch point with a twist to the left and in standard branch point configuration to the right. The net ± 1 twist on the horizontal edge is indicated by a cross-over closer to the vertex. The cross-over at the centre of the edge indicates the odd number of half-turns in the helical structure of the edge. The connections of the strands in figure 5(*a*) can be considered equivalent to those presented in figure 5(*b*) as indicated by the coloured strands. Choosing a branch point with a twist instead of a branch point of type A hence results in an annihilation of the extra twist on the edge. As each edge is incident to two faces, this change results in an 'orientable embedding' of the double helical structure along both pentagons that are boundaries of these two faces. This change is completely symmetric, whether it appears at the left or the right vertex incident to the edge. We indicate this change by placing a 'bead' on

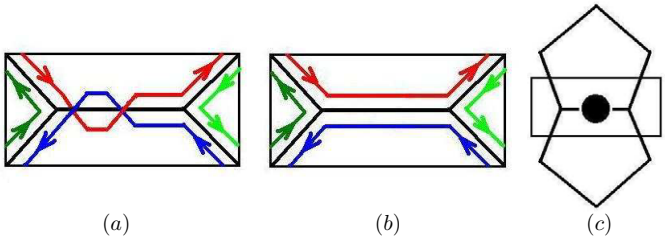

Figure 5. (*a*) A strand connection corresponding to the vertex type of a branch point with a twist indicated with a cross-over closer to the vertex to the left and in standard branch point configuration to the right. The cross-over at the centre of the edge indicates the odd number of half-turns in the helical structure of the edge. (*b*) An equivalent strand connection along an edge with two vertices in standard branch point configuration. Therefore the odd number of half-turns on the edges of the dodecahedron can be considered annihilated by a replacement of the standard branch point configuration by a configuration with a twist. (*c*) Schematic representation of the configuration shown in (*a*). The filled circle is called a *bead*.

the edge as indicated by figure 5(*c*). As there are 12 faces in the dodecahedron, the minimal number of changes ('placements of beads') needed to provide an orientable embedding of the whole double helical structure is six. There are several non-equivalent ways to introduce this change. We determine all such orientable embeddings of the DNA helical structure inside the cage and prove that there are only five symmetrically non-equivalent possibilities.

These inequivalent possibilities of placing beads on the edges of the dodecahedron such that precisely one edge per pentagonal face is decorated by a bead are obtained as follows. Without loss of generality, we place the first bead on any of the edges, because they are all equivalent by symmetry. We label this edge, and hence the bead on this edge, as 1; see figure 6. To keep the number of beads at a minimum, no other edge of the two pentagons labelled A and B can have a bead. Since it does not matter in which order pentagons are considered, we can next concentrate on the pentagon labelled C. Edges labelled 2 and 4 are equivalent by symmetry and hence lead to equivalent configurations. We therefore consider only the possibility of placing a bead on edge 2 or edge 3, and treat each case separately. A bead on edge 2 'covers' pentagons C and D in figure 6. Since every pentagonal face has to have a bead on one of its edges, we can, without loss of generality, look at pentagon E next. Due to the beads already present there is no symmetry, and thus all edges 5 to 8 lead to different solutions. There are ten solutions in total, denoted S1 to S10 in figure 7. We next concentrate on edge 3 instead of edge 2, hence pentagons A, B, C and I contain an edge with a bead. Up to symmetry arguments, there are three different ways to complete this bead game, labelled S11 to S13 in figure 7.

We investigate whether any of these 13 solutions can be symmetrically mapped onto another. To do so, we observe that different bead configurations can be classified according to different 'bead paths'. We call any path along edges of the decorated dodecahedron a *bead path* if, starting and ending on a beaded edge, it alternates along non-beaded and beaded edges. There are the following four scenarios of bead paths among the solution set:

Type I. All beads lie on a closed (cyclic) bead path (corresponding to solutions S1, S2, S4, S9).

Type II. All beads lie on a non-cyclic (open) bead path (corresponding to solutions S3, S5, S7, S8, S10, S12).

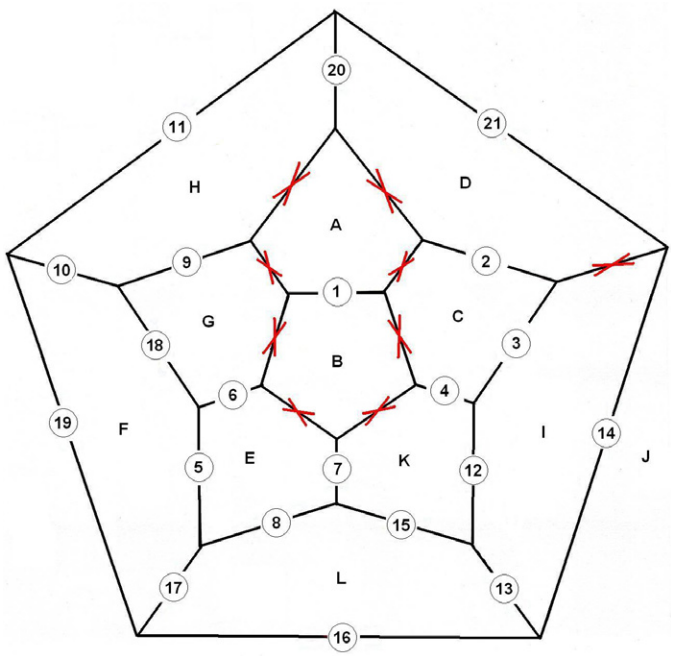

Figure 6. Labelling system corresponding to figure 7. Symbols (letters, numbers) identifying the pentagons follow the first branch of the solution tree in figure 7.

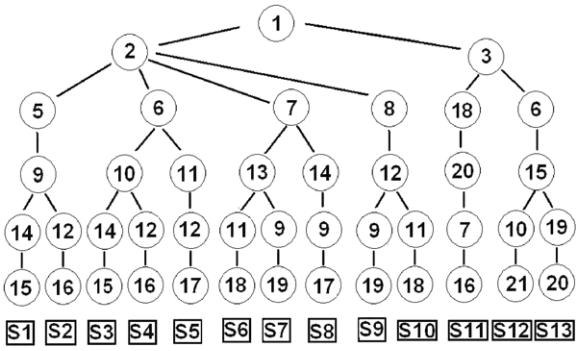

Figure 7. The tree encoding the 13 solutions of placing six beads on the dodecahedral edges such that each pentagon has precisely one bead. Only solutions S1, S2, S3, S6 and S11 are inequivalent.

Type III. All beads lie on three disjoint bead paths (with two beads each) (corresponding to solutions S6, S13).

Type IV. All beads lie on six disjoint bead paths one edge in length (corresponding to solution S11).

Bead placements corresponding to a given type (I–IV) are inequivalent to arrangements of any other type, because any symmetry would map a path onto another path, and a cycle onto another cycle. Hence an equivalence may appear only within a given type. We start with type I. Solutions S1 and S9 are mirror images of each other (obtained by turning the sphere inside out,

i.e., they have different helical structures but are otherwise identical). We therefore consider them as equivalent. Solutions S2 and S4 can be considered equivalent as they correspond to the closed path that divides the 12 pentagons of the dodecahedron into two identical sets of six. However, S1 and S2 are not related by symmetry, and there are hence two distinct solutions of type I, represented by S1 and S2. Solutions S3, S5 and S8 represent mutually symmetric open paths, while S7, S10 and S12 represent mutually symmetric paths, but of opposite orientation to S3, S5 and S8 (again obtained by turning the sphere inside out). Hence, we can consider all these options as equivalent, and so there is only one (up to symmetry) arrangement of beads producing a non-cyclic path, represented by S3. The two arrangements of type III corresponding to solutions S6 and S13 can be mapped one onto another. So there is only one solution of this type, represented, say by S6. Finally, S11 is the only solution of type IV. It corresponds to an equidistant distribution of beads on edges and is in that sense the 'most symmetric' solution of all.

Every bead configuration translates into an orientable embedding of the DNA duplex strands in the dodecahedron. The five inequivalent bead configurations and the corresponding DNA embeddings are depicted in figure 8.

Each of these DNA embeddings has 14 vertex configurations of type A and six vertex configurations of the branch point with a twist type in figure 2(*b*). The DNA embeddings corresponding to solutions S2 and S11 result in six distinct strands (figures 8(*d*) and (*e*)), the embeddings corresponding to solutions S1 and S3 in four strands (figures 8(*b*) and (*c*)), while the embedding corresponding to solution S6 in two strands (figure 8(*a*)). In order to construct a cage from a minimal number of DNA strands, the configurations from figures 8(*b*)–(*d*) are further analysed. By lemma 2.1, if a vertex configuration of type A (A1 or A2) obtained by hybridizing three separate strands is replaced by a vertex configuration of a branch point with a twist (figure 2(*b*)), then the number of strands meeting at the vertex reduces to one. Therefore, a replacement is chosen at vertices where disjoint sets of strands meet. In the case of six separate strands (i.e., the cases of S2 and S11) we need to choose two vertices for replacement, while for the case of four separate strands (i.e., the cases of S1 and S3) only one replacement is needed. For cases S1 and S11 this configuration change is shown in figure 9. In figures 9(*a*) and (*c*) the exchanged vertices are circled, and in figures 9(*b*) and (*d*) the strands have been connected as a result of the vertex replacements.

The replacement of a branch point by a branch point with a twist results in a change of the number of half-turns of the DNA strands, because these replacements add or remove a half-turn in the duplex structure. If the edges are equilateral and are rigid duplexes, i.e., have hybridization going up to the vertices, then there is no flexibility of the strands at the vertices, and the orientation of the strands does not allow such replacements of the vertex structure. The replacements shown in figures 9(b)and (d), resulting in a unification of the strands, are therefore obtained under the assumption that the strands at the vertices of the cage are either flexible or the lengths of the edges differ by a few nucleotides. In that case, a change in the connection of the strands does not interfere with the embedding of the double helical structure.

By lemma 1 in [20] with any other change of the connection of the strands at the vertices, the number of strands cannot be further reduced. In fact, the results in [20] show that the minimal number of strands to assemble a dodecahedron is 2. Figure 10(*a*) shows the two-strand configuration corresponding to solution S6. All vertices of this configuration are branch points or branch points with a twist, and the number of branch points with a twist is smaller than in those shown in figure 8. Therefore, if a single-stranded configuration of the cage is desired, the last two strands need to be 'unified' in another way. We propose that these two strands are unified by using a hairpin along one of the edges as shown in figure 10(*b*), connecting it with the strand of the incident vertex with a short single-stranded 'sticker strand'.

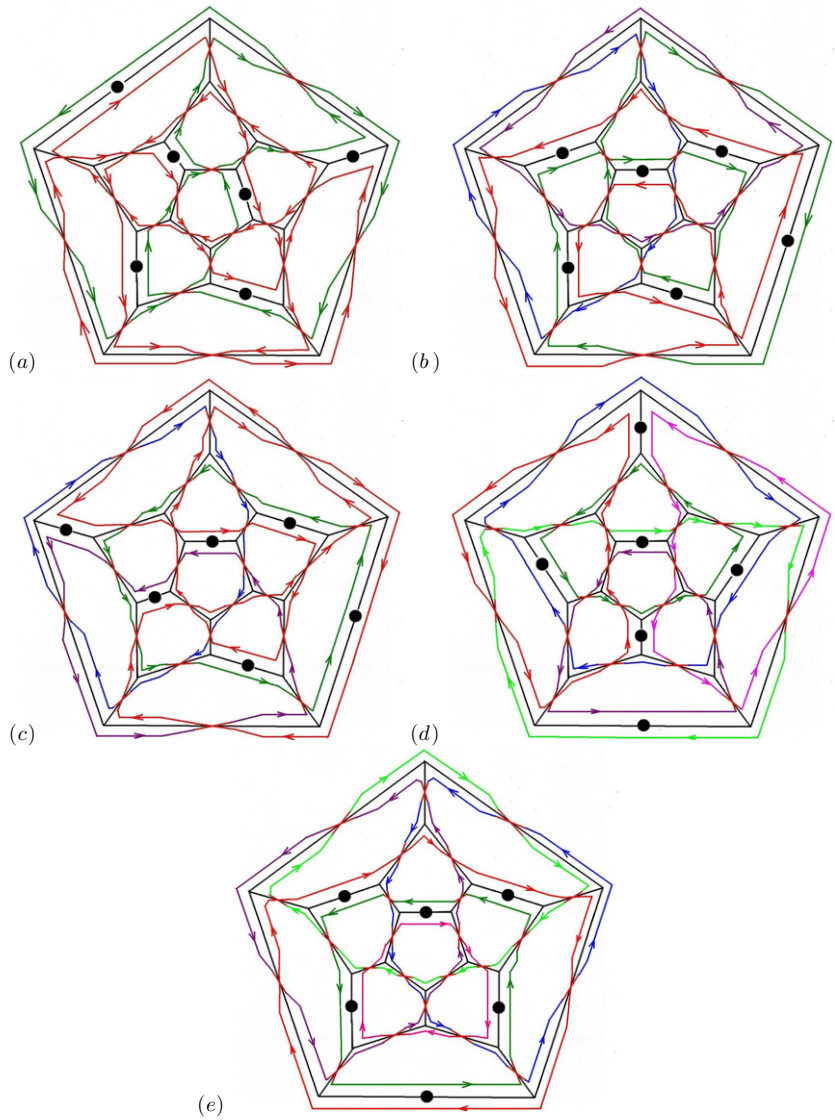

Figure 8. Embeddings of the DNA duplex structures in a dodecahedral cage corresponding to the inequivalent bead configurations. The bullets indicate the placements of beads, i.e., the edge containing an even number of half-turns. The configurations correspond to (*a*) solution S6 with two separate strands; (*b*) solution S1 with four separate strands; (*c*) solution S3 with four separate strands; (*d*) solution S11 with six separate strands; and (*e*) solution S2 with six separate strands.

3.2. Even number of half-turns per edge

We now consider the case of double-stranded DNA embeddings into dodecahedral cages with an even number of half-turns per edge. In these cases, there are no additional twists on the edges, because every edge corresponds to a double-stranded segment of about $11k (k \in \mathbb{N} \setminus \{0\})$ nucleotides. As before, we start with a planar representation of the dodecahedron and choose an orientation of the surface (neighbouring faces obtain opposite orientations, see for example

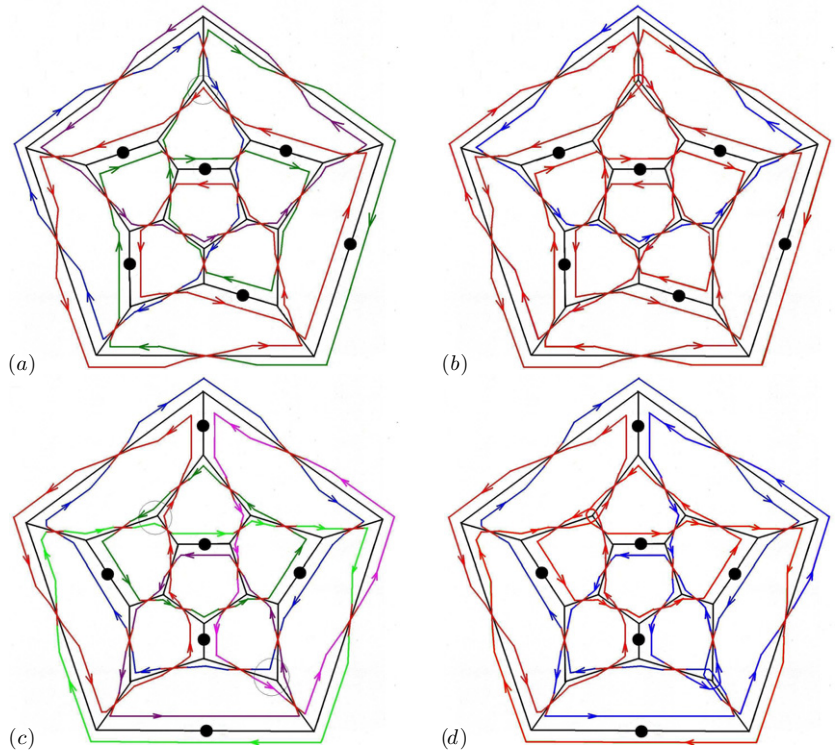

Figure 9. (*a*) Showing a possible vertex in the S1 configuration that may be replaced by a branch point with a twist in order to unite three of the strands using lemma 2.1. (*b*) The resulting configuration of a replacement of a branch point by a branch point with a twist in (*a*), provided there is flexibility of the strands at the vertex such that no additional twist is introduced. (*c*) Showing two possible vertices in the S11 configuration that may be replaced by a branch point with a twist. (*d*) The configuration resulting from (*c*) after replacement of the two encircled vertices by branch points with a twist.

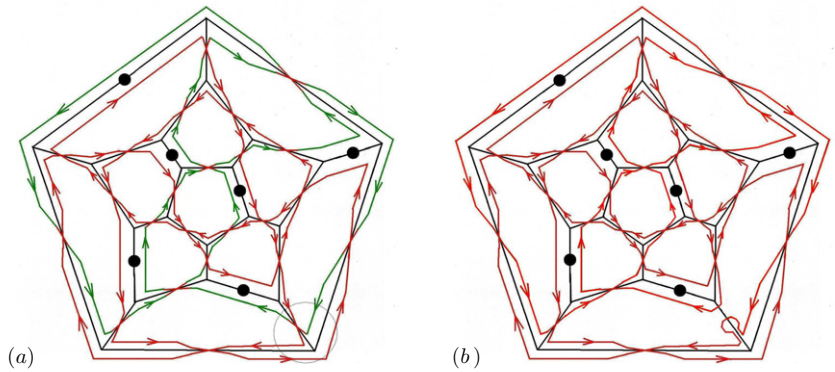

Figure 10. (*a*) Embedding of a duplex DNA structure corresponding to the solution S6 with two strands. (*b*) A hairpin extension is required at one edge, together with a sticker strand that connects it to the strand of the adjacent vertex, to obtain a one strand construction.

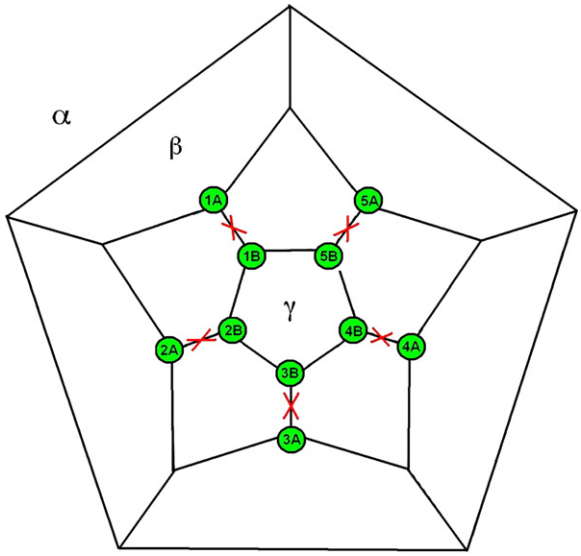

Figure 11. Via a replacement of five branch points by branch points with a twist, a net results of five extra twists (indicated by red crosses) is imposed on the overall structure, keeping the number of helical half-turns around each pentagon even. Green circles show the potential locations of the branch points with a twist. They must be located on one of the vertices incident to an edge marked by a cross, and options are labelled A and B.

[24]). We begin with a DNA embedding such that there is one strand per boundary of each face preserving the orientation (5′–3′ say). In this representation all vertex configurations are branch points. This DNA embedding leads to a configuration with 12 separate strands which is the maximal number of strands needed to assemble a dodecahedron [20]. Results in [20] imply that, given an appropriate arrangement, $2n + 1$ ($n \in \mathbb{N}$) separate strands can be connected into a single strand via a replacement of n ($n \in \mathbb{N}$) branch points of type A by branch points with a twist. Hence, for $n = 5$, a replacement of five branch points by branch points with a twist in the original 12-strand DNA embedding leads to a DNA embedding with two separate strands. The introduction of vertices with a twist must be done, in order to preserve the orientability of the strands, such that there are either precisely two extra twists per pentagonal face, or, no extra twists. Then, the number of half-turns in the DNA helix embedded around a pentagonal face remains even. However, this cannot be achieved via a replacement of only five vertices as we argue below.

To see this, note that the minimal way of marking five dodecahedral edges such that each face has either two marks or no mark at all, is the configuration shown in figure 11. To obtain a strand configuration with this distribution of twists as a result of replacements of branch points by branch points with a twist, replacements have to be made at one of the vertices incident to the marked edges, with alternatives shown as A and B in the green circles in figure 11. With every combination of five vertices ($1A \ldots 5A, 1B \ldots 5B$) the strand being a boundary of the outer face (labelled α in figure 11) cannot be unified with the other strands. Consider a combination of five vertices that includes one of the B-vertices in figure 11, $1B$ say. This places an extra twist on the edge connecting the vertices $1A$ and $1B$, hence vertex $1A$ cannot be replaced. The strand representing the boundary of the pentagonal face labelled β in this figure cannot be unified with the strands that are obtained by any combination of replacements of

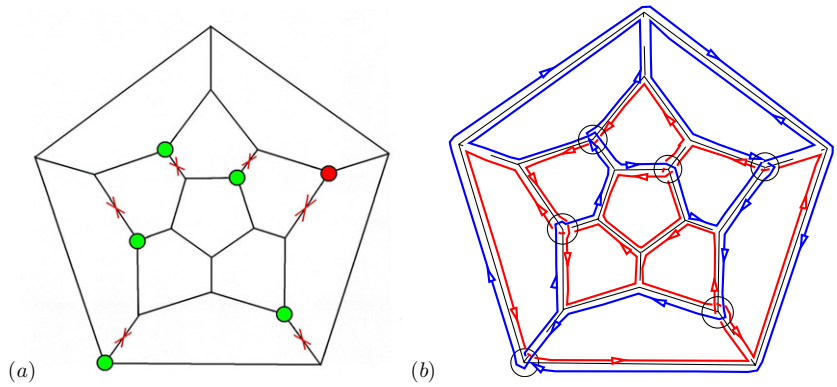

Figure 12. Scenario for non-flexible strand connections at vertex junctions. (*a*) A reduction to a two-strand configuration via a replacement of branch point vertices by branch points with a twist at the junctions marked by green circles. The replacement of the sixth vertex marked in red keeps the orientability of the strands within the structure. (*b*) The two-strand embedding after a replacement of the six vertices.

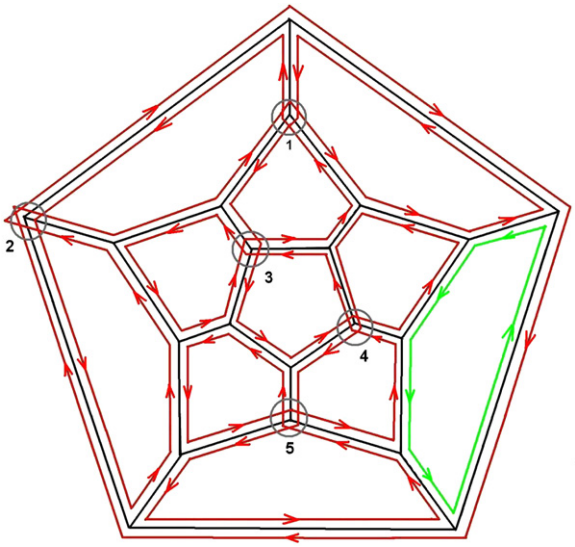

Figure 13. Scenario for flexible strand connections at vertex junctions. Any replacement of five branch points by branch points with a twist at places where three distinct strands meet leads to a two-strand configuration, for example a replacement at the circled vertices.

any four vertices from $\{2A, \ldots, 5A, 2B, \ldots, 5B\}$. This means that strands being boundaries of the central face γ, and those of the faces α and β, cannot be unified. Since the number of strands of a DNA embedding in a dodecahedral cage must be even, a combination of five vertex replacements, one of which is $1B$, leaves a DNA embedding with at least four distinct strands. Similarly, a combination of replacements corresponding to vertices $1A, \ldots, 5A$ does not affect the strand representing the boundary of face γ, and again, strands corresponding to boundaries of the faces α, β and γ cannot be unified.

It is possible to perform six vertex replacements such that the number of helical half-turns around each pentagon remains even. One such possibility is shown in figure 12(*a*). By

replacing each type A vertex indicated by a circle in figure 12(*a*) by a branch point with a twist vertex (see figure 12(*b*)), a DNA embedding into the dodecahedral cage with only two strands is obtained, which subsequently could be unified as mentioned before with a hairpin extension along one of the edges and a sticker strand. The configuration shown in figure 12(*b*) corresponds to (by vertex construction) the optimal solution.

For structures with flexible strand connection at the vertex junctions, any replacement of five vertices at places where three distinct strands meet (such as in figure 13(*a*)) leads to a configuration with two separate strands. An example is shown in figure 13(*b*).

4. Concluding remarks

In view of applications in bio-nanotechnology, we proposed here optimal arrangements of DNA strands in a dodecahedral cage such that the overall structure is obtained with a minimal number of strands. The strand connections at the vertex junctions can be flexible (meaning, there are mismatches of the bases close to the branch points) and non-flexible (meaning the strand hybridization are all the way up to the junctions and no mismatches occur). In the case of flexible connections, only two mismatches may be sufficient such that a branch point with a twist type of vertex configuration would not strain the dodecahedral structure. This was shown to be the case at least for the graph construction *in vitro* reported in [13]. Allowing flexible junctions at the vertices by adding non-hybridized thymines (see for example [13, 25]) allows for equilateral edges throughout the cage at the expense of a less rigid structure. On the other hand, small differences in the lengths of the edges permit a full duplex structure along the edges and results in a more rigid cage configuration. The choice of one over the other may be determined by the applications.

In order to engineer such a dodecahedral cage in bio-nanotechnology from the blueprint provided here, the three junctions have to be used as building blocks for assembly. In particular, as in [20, 26], the junctions must be equipped with sticky ends, i.e., single DNA strand extensions, that are engineered such that they hybridize with their Watson-Crick complements. After hybridization the strands may be ligated to enforce the edge connections. Although in applications it may be desirable to keep the cage with non-ligated edges, for example to facilitate an easier release of a cargo.

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