Russian Chemical Reviews **81** (2) 130 – 157 (2012) © 2012 Russian Academy of Sciences and Turpion Ltd

DOI 10.1070/RC2012v081n02ABEH004265

Principles of DNA architectonics: design of DNA-based nanoobjects

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Abstract. The methods of preparation of monomeric DNA blocks that serve as key building units for the construction of complex DNA objects are described. Examples are given of the formation of DNA blocks based on native and modified oligonucleotide components using hydrogen bonding and nucleic acid-specific types of bonding and also some affinity interactions with RNA, proteins, ligands. The static discrete and periodic two- and three-dimensional DNA objects reported to date are described systematically. Methods used to prove the structures of DNA objects and the prospects for practical application of nanostructures based on DNA and its analogues in biology, medicine and biophysics are considered. The bibliography includes 195 references.

I. Introduction

The Watson – Crick base-pairing rule, which underlies the interactions of nucleic acid (NA) molecules, determines the intensive use of NA in various molecular-biology applications related to the formation of stable and sequence-specific complexes [polymerase chain reaction (PCR), hybridization analysis, antisense approaches, *etc.*]. The programmed self-assembly of multicomponent DNA complexes the course of which is specified by the selection of the nucleotide sequences of the components has served as the main impetus for the formation of a fundamentally new NA application, namely, structural DNA nanotechnology or DNA architectonics. The works in this field are aimed at the design of structures of definite shape and spatial organization where DNA molecules serve as building blocks with programmed self-association behaviour.

DNA molecule is a unique object for the fabrication of nanostructures for a number of reasons:

— the structural parameters of complexes based on DNA chains (duplexes, triplexes, quadruplexes and so on) are known and are in the nanometre range;

— methods of synthesis of non-modified (native) NA molecules and their derivatives have been developed and automated;

— the thermal stability of DNA complexes as a function of the nucleotide sequence and external factors (the nature of the cations, NA-specific ligands, co-solvents and so on) has been comprehensively studied; the key principles of self-association of DNA strands have been determined, which is important for programming the shape of DNA objects and suppressing the formation of side products;

— procedures for the introduction of modifying components into any specified site of the DNA molecule with retention of its capacity for specific interactions have been developed; this can be used to increase the functionality of DNA structures;

— functions of many DNA-dependent enzymes have been discovered and described; they could be used both to design DNA objects (for example, DNA ligases are used for cross-linking of the building blocks to give more complex structures) and to verify their structures and remove side products (*e.g.*, nucleases);

— the objects based on biological molecules possess a number of unique properties (biocompatibility and biodegradation), which opens up the prospects for their practical applications (*in vitro* and *in vivo*) in biology, medicine, *etc.*

Initially, native oligonucleotides and DNA duplexes served as building blocks in DNA nanotechnology. The design from only native NA poses some limitations on the shape of the resulting objects, as the double-stranded molecule (dsDNA) is a rigid, topologically linear structure. The persistent length of the DNA polymer is ~25 – 100 nm

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Received 16 August 2011
Uspekhi Khimii **81** (2) 130 – 157 (2012); translated by Z P Svitanko

depending on the composition and ionic strength of buffer solutions.[1] It is clear that linear DNA molecules present no considerable interest for the design of two- or three-dimensional architectures. Therefore, more and more often, DNA duplexes containing additional modified units that impart the missing flexibility to the dsDNA molecules are considered as the building blocks.

Two key trends can be distinguished in the structural DNA nanotechnology, in particular, the design of static nano- and microobjects and the design of dynamic structures (molecular machines) capable of controlled change in their shape and organization features. In terms of their structure, static DNA objects are classified into discrete (isolated structures of an invariable size) and extended periodic structures formed upon successive addition of identical or different monomer units.

A large number of original and review publications of the last decade consider numerous aspects of the design of DNA-based objects (see, for example, reviews [2–14]). However, most reviews are focused on particular stages of construction of the DNA structures. Some of the papers describe the monomer units used in the DNA nanoarchitectonics for the formation of complex DNA items; the discrete and periodic static DNA objects and dynamic molecular DNA machines are described. Some reviews give examples of introduction of modified non-nucleotide residues that contribute to both structural and functional diversity of DNA objects.

This review surveys published data, mainly of the last two decades, concerning the principles of design of DNA structures starting from the selection of the constituent oligonucleotides and assembly of DNA blocks and ending with construction of static DNA objects from them and considering the prospects and the first steps of their practical applications. The state-of-the-art knowledge of monomeric structural DNA blocks of diverse shapes made of native and modified oligonucleotide components and the DNA objects constructed using these blocks is fully systematized. The effect of the pattern of self-programmed monomer assembly and characteristics of the monomer units such as rigidity (inherent in the DNA backbone) and structural flexibility (arising upon introduction of modifying elements) on the efficiency of formation of the predicted NA objects is considered.

II. Formation principles of DNA blocks

The primary task of researchers who intend to construct DNA objects is to determine the basic structure of the DNA blocks, *i.e.*, monomeric building units that can serve as the basis for the formation of the desired multicomponent object. Often, for the formation of a particularly shaped DNA object, it is necessary first to design DNA blocks from unusual components: bent or branched NA. The connection of the obtained DNA blocks to one another produces the desired two- and three-dimensional DNA structures. A gamut of typical blocks based on native oligonucleotides that can largely diversify the sizes and shapes of DNA nanoarchitectures has been developed to date.

1. Branched DNA blocks based on native oligonucleotides

Branched DNA structures occur in living systems as short-lived intermediates involved in replication, recombination and repair. Due to the symmetry of nucleotide sequences relative to the branching point, such structures are not sufficiently stable (Fig. 1 *a*). The structural DNA nanotechnology is thought to be started in 1982 where Seeman [15] attempted to design topologically and geometrically diverse DNA objects on the basis of branched Holliday junctions formed upon the recombination of nucleic acids. Seeman and co-workers [16] were the first to obtain stable four-way branched analogues of the Holliday junction structures. The uniqueness of their shape is ensured by the choice of the synthetic oligonucleotide sequences forming them (Fig. 1 *b*). By analogy, three-arm (3A),[17,18] four-arm (4A) (Fig. 2 *a*),[16,19,20] five- and six-arm (5A and 6A)[21] and eight- and twelve-arm (8A and 12A) synthetic junctions,[22] the arms representing dsDNA fragments, have now been

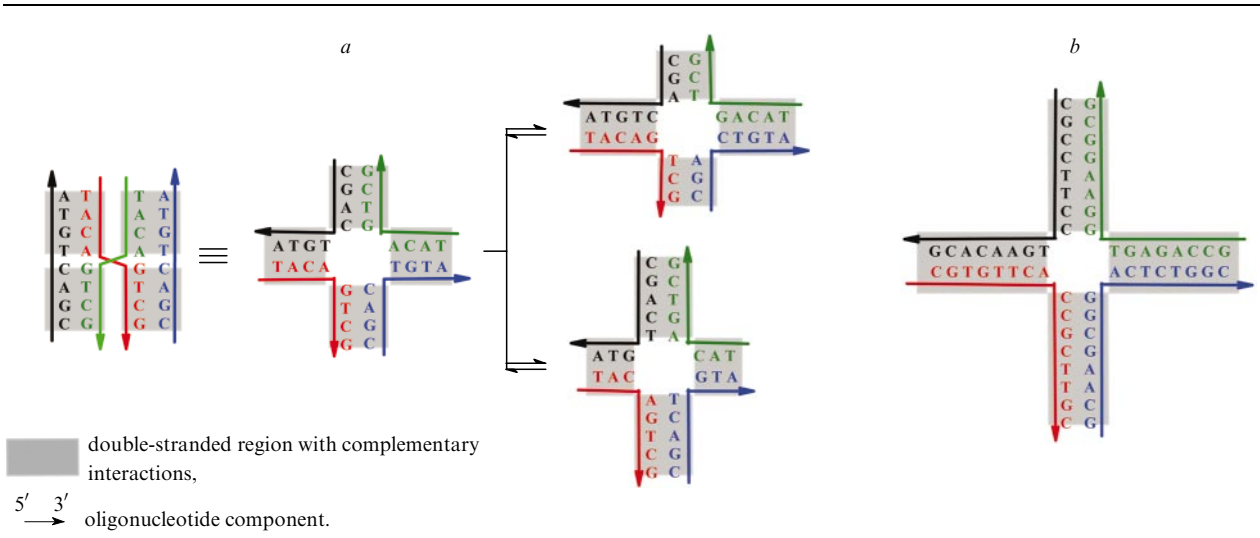


Figure 1. Holliday junctions.[15]
Migration of the branching point in the Holliday junction formed upon recombination (*a*). Structurally fixed four-way branched analogue of the Holliday structure (*b*).

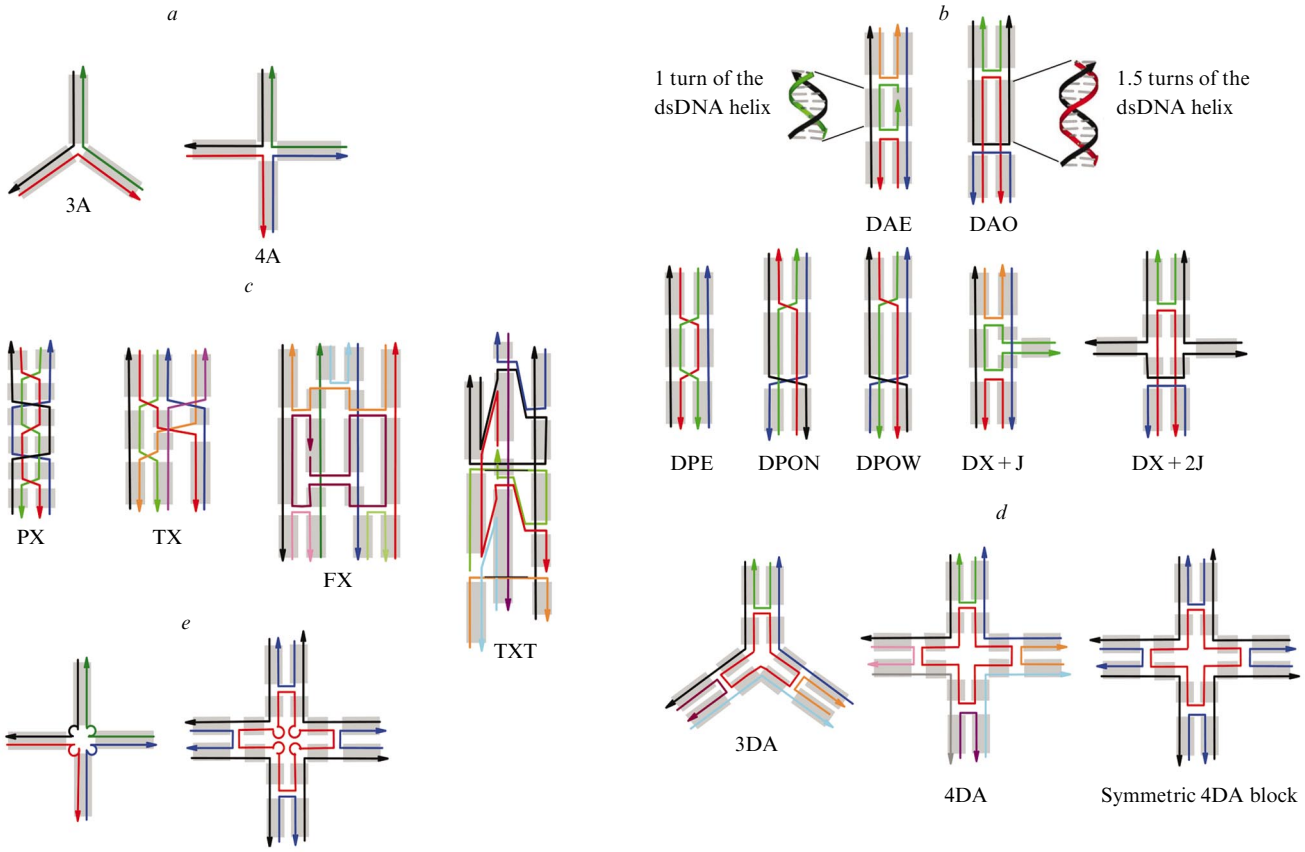


Figure 2. Branched DNA blocks based on native oligonucleotides (see text).[16–34]

designed. This type of structures is the simplest example of DNA blocks used in DNA architectonics.

Later, more rigid branched blocks were designed based on oligonucleotide systems. Unlike the A blocks containing one crossover point, they contained two crossover points separated by dsDNA regions (Fig. 2 *b*).[23–25] Double crossovers (DX) are subdivided into two classes according to the relative orientation of the dsDNA fragments that form them: either parallel (DP) or antiparallel orientation (DA). Each of the two crossover types is additionally classified according to the number of half-turns of the DNA helix between the junctions, this number being either odd (DPO and DAO) or even (DPE and DAE). The crossovers with odd number of half-turns are classified into those where the odd half-turn occurs along the major (wide) groove of the double helix (DPOW) and those where the odd half-turn occurs along the minor (narrow) groove (DPON). Figure 2 *b* shows the key types of DNA blocks based on double crossovers. The authors note that the obtained parallel crossovers do not have sufficient stability; they either dissociate or form multimers.[23]

Topologically, all of the DX blocks shown above are X-type structures with four DNA helices as arms. The DX blocks were used to form five- and six-way branched DNA blocks (DX + J and DX + 2J, respectively) (Fig. 2 *b*).[24, 25]

The native oligonucleotides were used to obtain more complex paranemic crossover (PX) patterns in which two parallel DNA duplexes form crossovers at every point possible, *i.e.*, each of the four DNA chains forms a curl over an integer number of turns (Fig. 2 *c*).[25–27] In addition, DNA blocks based on a larger number of parallel dsDNA paired through double crossovers were obtained. For example, triple crossovers (TX) made of three DNA helices [25] and crossovers made of four (FX) (Fig. 2 *c*), eight (EX) and twelve (TwX) dsDNA duplexes were constructed.[28, 29] In addition, the TX block was used to obtain a three-dimensional TXT block (Fig. 2 *c*).[30]

The DA (double arm) blocks, which are similar to the A blocks but are more rigid, are branched structures with DNA arms, each comprising two parallel DNA duplexes joined by a crossover point. The formation of these three-, four-, five- and six-stranded patterns (3DA, 4DA, 5DA and 6DA) was demonstrated (Fig. 2 *d*).[31–34] These complex blocks have structural stability and rigidity, unlike the A blocks; however, their construction requires more oligonucleotides. Indeed, 3A, 4A, 5A and 6A blocks are formed with participation of 3, 4, 5 and 6 oligonucleotides, respectively, whereas the construction of the 3DA, 4DA, 5DA and 6DA blocks requires hybridization of 7, 9, 11 and 13 components, respectively. Mao and co-workers [32–34] proposed a strategy for the preparation of symmetric DA

blocks (see Fig. 2 *d*), which reduced the number of constituent oligonucleotides to three and, hence, made the preparation process less labourious.

Usually, for the construction of complex DNA objects from branched blocks, single-stranded fragments are introduced at the branching point for producing additional structural mobility (Fig. 2 *e*).[33, 34] The length of the single-stranded regions may vary depending on the tasks of DNA nanodesign.

The construction principles of the above-described multibranched DNA blocks are based on several rules: minimization of the symmetry of the sequences of particular oligonucleotide components relative to the branching point, *i.e.*, the need to ensure an 'orthogonal' arrangement of the NA sequences of the system components; assembling of the DNA block from oligonucleotide constituents taken in a strictly stoichiometric ratio.[23, 24]

Note that the monomeric blocks can be assembled in more complex structures of a desired shape. A simple and widely used form of cohesion between DNA blocks is the interaction of their sticky ends. The blocks are initially formed in such a way that they contain single-stranded regions complementary to each other, and through these regions they are assembled into the structure of a higher organization level. By varying the number and positions of sticky ends, one can specify the direction of assembly and hence the bottom-up organization of one-, two- or three-dimensional DNA objects. An advantage of using sticky ends is the possibility of covalent cross-linking of the blocks, for example, using DNA ligases.

2. DNA blocks incorporating additional structural parts

The formation of the above DNA blocks is based only on pairing of oligonucleotides and their fragments. Other approaches to the organization of branched DNA blocks are related to the use of additional structural parts, for example, proteins or RNA fragments and the residues of organic molecules, which function as molecular staples and thus favour the assembly of DNA blocks with non-linear shapes.

a. DNA blocks obtained using affinity interactions of nucleic acid molecules

In the construction of branched DNA blocks from native oligonucleotides, specific types of interactions of DNA molecules may serve as the branching point. Thus a hydrogen bond network arises between four guanine residues located in one plane and, hence, oligo- and polyguanine fragments form so-called G-quadruplexes under controlled conditions.[35] On the basis of G-quadruplexes, it was proposed to introduce branching points into multimolecular associates requiring the presence of 4A-like blocks (Fig. 3 *a*).[36–38]

RNA fragments are used as staples in the formation of DNA nanostructures; these can be easily introduced into a hybrid NA block during its automated synthesis or upon interaction of the nucleic acid chains to form the DNA–RNA hybrid complex. When there are specific RNA domains, it is possible to resort to unique types of intermolecular interactions inherent in RNA molecules for assembling the supramolecular complexes. For example,

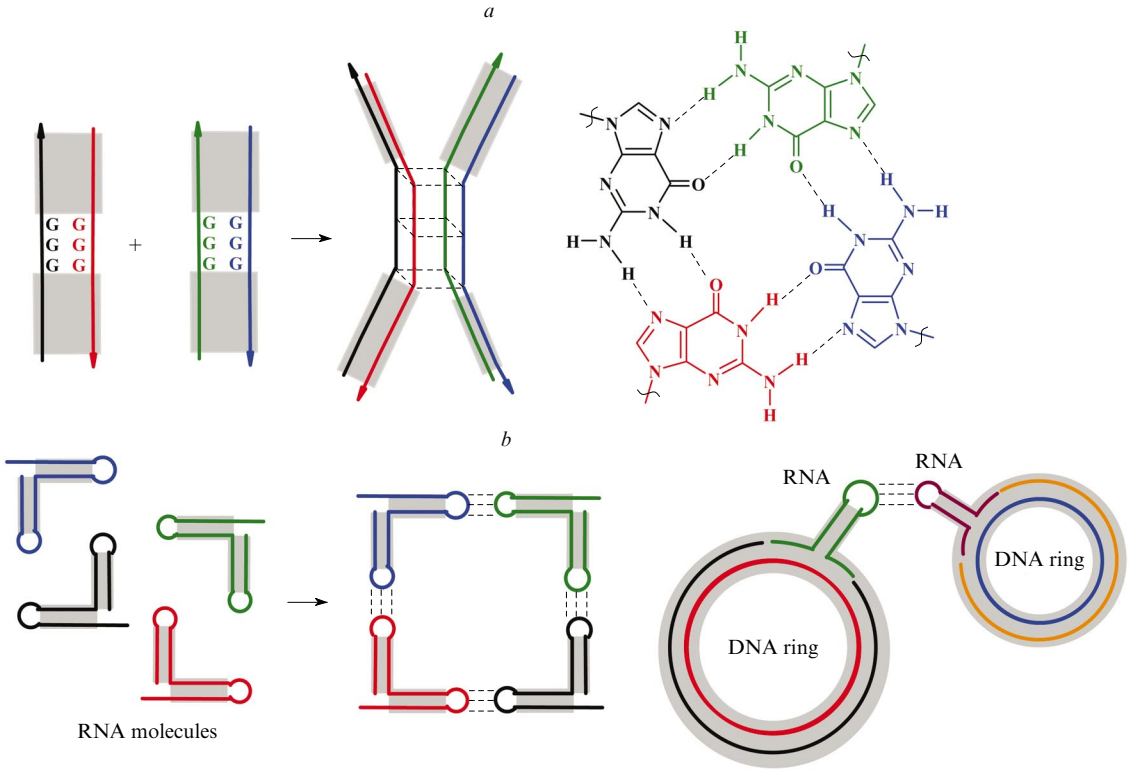


Figure 3. Formation and association principles of DNA and RNA blocks using affinity interactions of nucleic acid molecules: G-quadruplexes (*a*),[36–38] kissing interactions of RNA molecules (*b*).[41–43, 46]

strong binding of RNA molecules may arise upon contacting of the tetranucleotide GAAA sequence in the hairpin loop with the corresponding 11-nucleotide RNA receptor,[39–41] upon interaction of the loop fragments of two hairpin structures involving 5–7 complementary base pairs (so-called kissing interactions)[42,43] or other unique RNA fragments.[44] Using the interactions of RNA hairpins, extended linear and two-dimensional planar structures and ring RNA objects were obtained (Fig. 3 *b*).[41–43] The design of diverse structures based on RNA molecules was called RNA tectonics.[41,45] The specific RNA/RNA interactions can also be utilized for the deliberate assembly of DNA nanostructures. For example, a method for connection of a pair of ring dsDNA structures having single-stranded gaps was proposed. Linking of special RNA fragments to such single-stranded sites in each of the DNA blocks ensures cohesion of these blocks through kissing interactions of two RNA hairpins (Fig. 3 *b*).[46]

Thus, the use of other nucleic acid-specific types of binding together with the Watson–Crick base pairing may prove to be a potent tool for the preparation of DNA blocks with diversified shapes.

b. DNA blocks based on protein–DNA complexes

The adjustment of the NA fragment to be recognized to the enzyme active site, which is often accompanied by bending of the linear DNA helix, is known to be an important stage of enzymatic processes that occur in living systems and involve DNA molecules (transcription, replication, regulation, *etc.*).[47–51] Apart from enzymes, the proteins functioning as regulation factors (activators, inhibitors) of cellular processes can also be responsible for bending and stabilization of the unusual shape of a DNA chain. An example of proteins that cause the DNA helix to bend by 90° is the catabolite activator protein (CAP).[52,53] A comparable bending of dsDNA is induced by binding to the *Lac* repressor protein,[54] the integration host factor (IHF)[55] the TATA-binding protein[56] and so on. A somewhat smaller bending was described for the cases of DNA interaction with the *cro*-repressor,[57] histone-like proteins (HU)[58] and some other proteins.

It was noted above that the branched DNA intermediates (Holliday junctions) detected in living systems are dynamically unstable, and in the native form they cannot be used for the design of DNA objects. However, it was shown that the spatial organization of the Holliday structures can be stabilized in the presence of the RecA protein.[59] The structure thus obtained does not show dynamic changes even upon long-term incubation (~6 h) at 37 °C. At 56 °C, about 60% of the initial Holliday structures are retained after the same period of time.

Yet another method for the preparation of branched DNA blocks is based on the use of highly specific biotin – streptavidin interaction. Streptavidin is a tetrameric protein with one biotin-binding site in each subunit. Thus, theoretically, a protein molecule can bind four biotinylated DNA fragments to form an X-shaped structure with the centre (branching point) located on streptavidin. However, it was shown that upon interaction with a fivefold excess of 5′-biotinylated dsDNA of ~100 –200 bp length (bp are base pairs), streptavidin mainly binds not more than two NA molecules. The associates bearing three and four DNA fragments are formed with low efficiency, 10% and 5%, respectively, of the total number of the biotin – streptavidin complexes formed. In the authors' opinion, destabilization of protein conjugates is caused by both the electrostatic repulsion of negatively charged DNA fragments and steric hindrance.[60] Nevertheless, Tian *et al.*[61] presented evidence that incubation of 2000 bp long biotinylated PCR fragment with streptavidin affords branched DNA blocks containing two, three and four branches in comparable amounts. An advantage of using DNA–streptavidin associates for preparing more complex items is that the protein molecules can serve in this case not only as branching points but also as frames for attachment and selective positioning of additional functional residues.

Thus, DNA fragments complexed with protein molecules can change their native conformation by forming stable branching and/or bending points.

c. DNA blocks based on modified oligonucleotide components

The introduction of organic derivatives into the regular nucleic acid backbone may give rise to additional functionality without deteriorating the sequence-specific behaviour. Various modifications open up the way for deliberate change of not only the physicochemical properties of NA complexes but also the spatial organization of blocks based thereon.

The structural modifications of nucleotide components used most often in DNA architectonics can be classified into three groups:

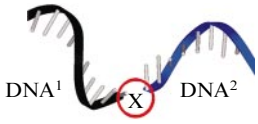
— modifications introduced into the oligonucleotide derivatives during the automated synthesis (Table 1);

— modifications providing post-synthetic assembly of oligonucleotide derivatives to form the desired DNA block and/or DNA nanostructure (Table 2);

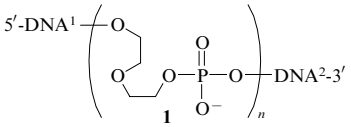
— modified NA fragments in the coordination complex with a metal cation (Table 3).

Currently, a large variety of organic phosphoramidite synthons used in the automated synthesis of oligonucleotides apart from natural nucleotide monomers have been reported. These synthons can be represented by linkers that connect two native oligonucleotide fragments or by more complex branching modifications connecting three and more nucleotide chains. The DNA blocks based on even simple modifications connecting two NA fragments are promising for the preparation of complex items because they can bend the dsDNA backbone.[62,63] For example, the bending angle of the DNA duplex upon the introduction of internucleotide linkages based on oligoethylene glycol (**1**) and oligomethylenediol (**2**) phosphodiesters, which form flexible loops in the modified double-stranded DNA fragment, was estimated quantitatively (see Table 1). The possibility of control over the scale of local perturbation of the DNA duplex by varying the nature and the size of linkages was demonstrated. This local perturbation is accompanied by the change in the angle between the DNA arms that go from the modification point from 106° to 164°.[62] The introduction of a modification based on 1,3-bis(4-hydroxyphenyl)benzene residue (**3**) into one oligonucleotide component of the DNA duplex would promote most likely the formation of a bent DNA block with the dsDNA arms being mainly arranged at a 120°.[63] However, note that the use of modified residues is distinguished by not only the possibility of bending dsDNA through a particular specified angle but also by imparting dynamic flexibility to complexes based on the corresponding NA derivatives, which is missing for the native analogues. If it is necessary to prepare a

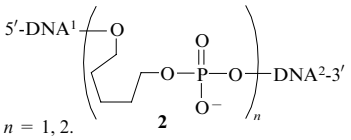
Table 1. Modifications of oligonucleotides introduced during automated synthesis and giving rise to dsDNA bending and/or branching.



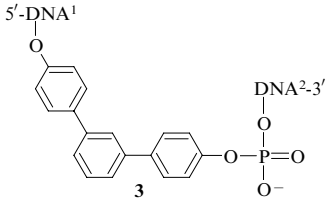
Oligoethylene glycol phosphodiester [62]



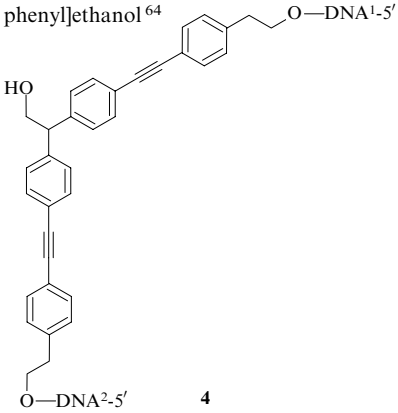
Oligomethylenediol phosphodiester [62]

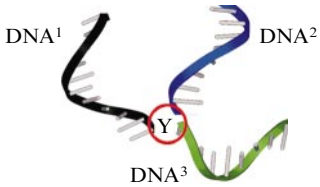


1,3-Bis(4-hydroxyphenyl)benzene [63]

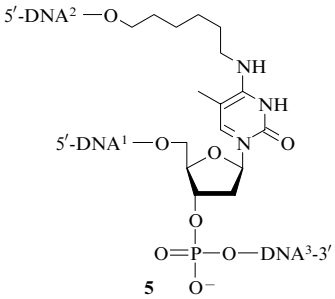


2,2-Bis[4-(2-hydroxyethyl)phenylethynyl-phenyl]ethanol [64]

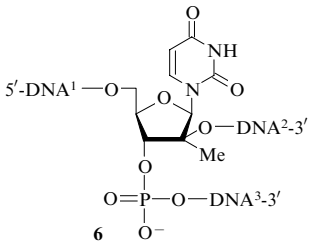




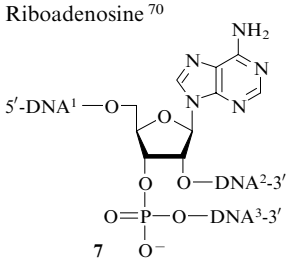
N(4)-(6-Hydroxyhexyl)-5-methyl-2′-deoxycytosine [65–68]



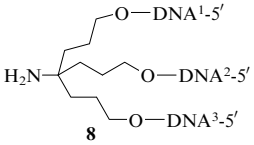
(2-Methyl-β-D-arabinofuranosyl)uracil [69]



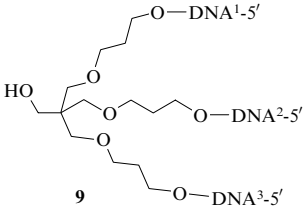
Riboadenosine [70]



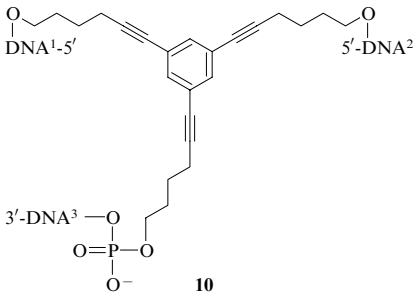
Tris(3-hydroxypropyl)aminomethane [71]



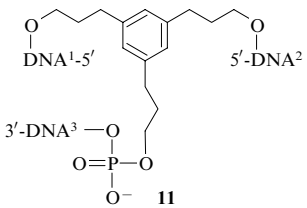
Tris(3- hydroxypropyloxymethyl)ethanol [72]



1,3,5-Tris(6-hydroxyhexynyl)benzene [73]

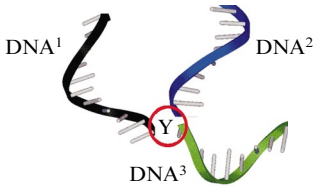


1,3,5-Tris(3-hydroxypropyl)-benzene [74]

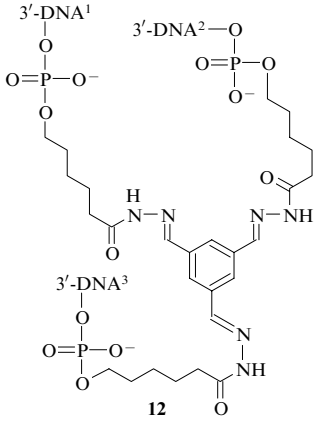


Note. The names refer to compounds used to prepare linkers X and Y.

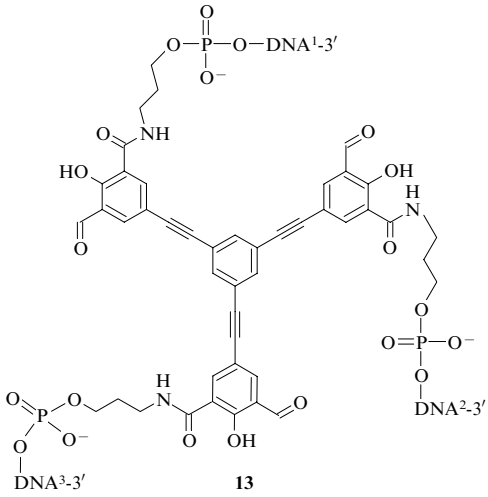
Table 2. Modified residues providing the post-synthetic assembly of oligonucleotide derivatives to branched DNA block.



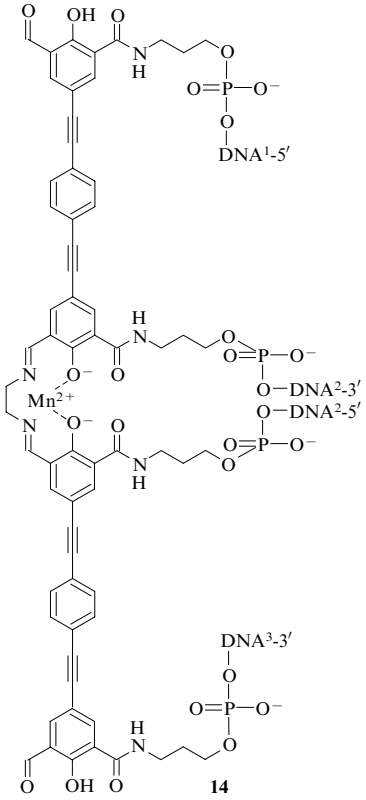
Linker based on 1,3,5-trishydrazobenzene [75]

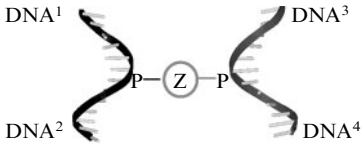


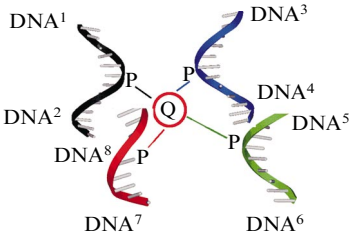
Linker based on 1,3,5-triethynylbenzene [76, 77]



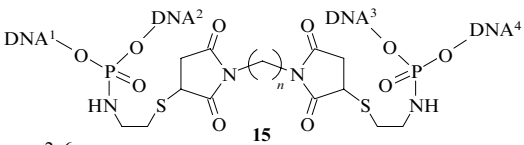
Ethylenediimine chelate-containing linker [76, 77]





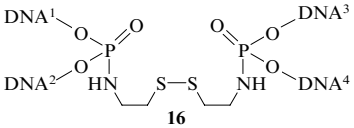


Bismaleimide bridge [78]

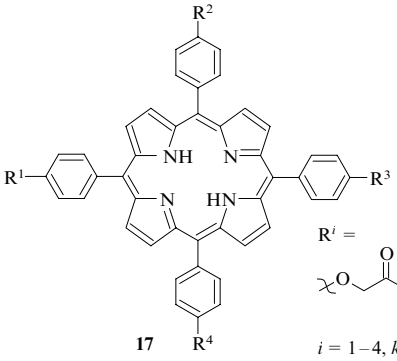


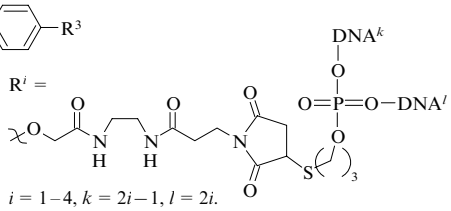
n = 2, 6.

Disulfide bridge [79, 80]



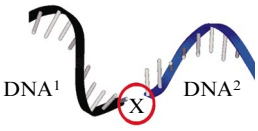
Linker based on porphyrin [81, 82]



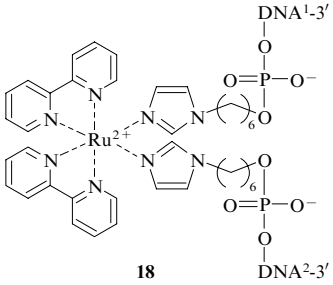


i = 1–4, k = 2i − 1, l = 2i.

Table 3. Modifications of oligonucleotides based on chelating residues providing association of NA fragments in the coordination complex with a metal cation.

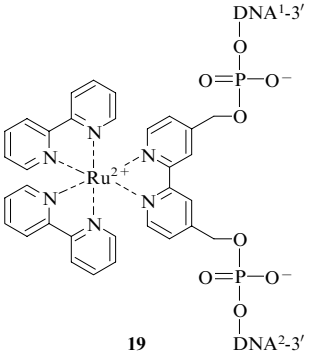


$[Ru^{II}(bipy)_2(Imd)_2]^{2+}$ (Ref. 83)



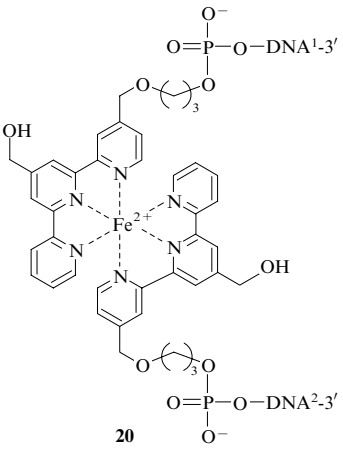
18

$[Ru^{II}(bipy)_3]^{2+}$ (Ref. 84)

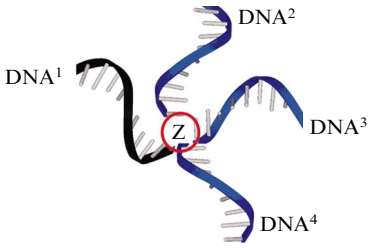


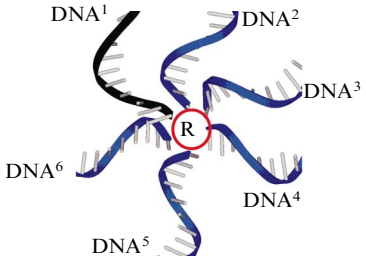
19

$[Fe^{II}(tpy)_2]^{2+}$ (Ref. 85)

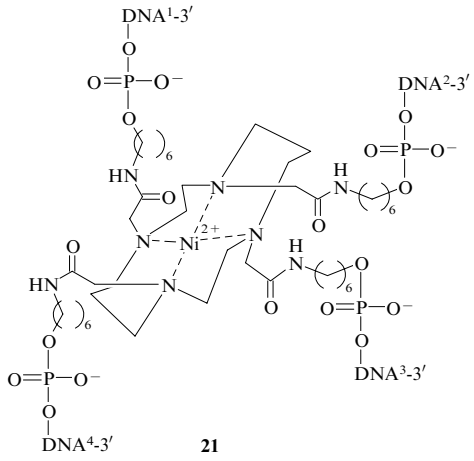


20



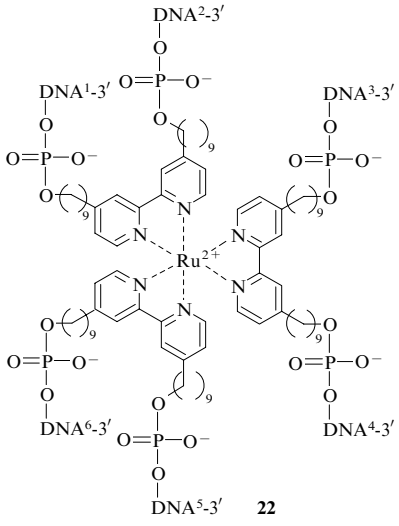


$[Ni^{II}(tactd)]^{2+}$ (Ref. 86)



21

$[Ru^{II}(bipy)_3]^{2+}$ (Ref. 87)



22

derivative with a pair of free 5′-terminal sites, DNA components are synthesized using, for example, a solid support bearing 2,2-bis[4-(2-hydroxyethyl)phenylethynylphenyl]ethanol (**4**).[64]

The synthesis of branched oligonucleotides from special synthons is performed by standard protocol: the nucleotide chain grows up to the position of the modifier, and after the modifier has been introduced, the synthesis of the remaining identical or different oligonucleotide fragments is continued simultaneously or consecutively. The branched oligonucleotide architectures are obtained using compounds that contain two or more hydroxy groups suitable for the addition of the subsequent nucleotide units. The selection of O-specific protecting groups in these synthons determines the type of resulting branched oligonucleotide derivatives. In the synthesis of architectures with identical fragments, compounds with identical protecting groups are used after the branching point.[71,88] To obtain the product with different sequences, a modifying synthon with chemically different O-protecting groups that can be selectively removed (orthogonal) are to be introduced after the branching point.[65,73]

Two key types of branching synthons can be distinguished: nucleotide derivatives and non-nucleotide organic compounds. The first type of phosphoramidites is exemplified by compounds based on *N*(4)-(6-hydroxyhexyl)-5-methyl-2′-deoxycytosine (**5**),[65–68] 1-(2-methyl-β-D-arabinofuranosyl)uracil (**6**)[69] and riboadenosine (**7**);[70] their introduction into the oligonucleotide sequence being synthesized produces three-way junctions. To protect the additional hydroxy group that serves for attachment of the third NA sequence, the levulinoyl moiety is used in the case of the n-hexanol OH function at the cytosine N(4) atom and the *tert*-butyldimethylsilyl protection is used in the case of the riboadenosine 2′-OH group. Three-way branched oligonucleotides are obtained most often using synthons that bear the following O-protecting groups: dimethoxytrityl, levulinoyl, *tert*-butyldimethylsilyl or allyloxycarbonyl group.

There exists a broad range of non-nucleotide monomers that are incorporated into the growing oligonucleotide chain to enable its branching. Such synthons were prepared from derivatives of tris(3-hydroxypropyl)aminomethane (**8**),[71] tris(3-hydroxypropyloxymethyl)ethanol (**9**)[72] and so on. These monomers are suitable for the design of NA dendrimers that contain multiple numbers of the same oligonucleotide. Structure junctions of three different oligonucleotide fragments were obtained, for example, using synthons based on triols, *viz*., 1,3,5-trisubstituted benzenes **10** and **11**.[73,74]

For increasing the yield of branched oligonucleotide derivatives, the following conditions should be fulfilled: the polymer support should have large pores (1000–2500 Å); a linker should be present between the organic residue and the oligonucleotide sequence;[63,65,67,88] in some cases, efficient incorporation of the modifying residue is possible only at a sufficient distance from the polymer. An NA sequence at least 20 nucleotides long can serve as such a linker.[67]

The second approach to the design of branched NA blocks is post-synthetic assembly of the pre-synthesized linear fragments (see Table 2). For example, three oligonucleotide derivatives bearing 5′-hydrazide-modified residue can be conjugated to give tris-hydrazone linker **12**.[75] The introduction of the salicylaldehyde residue at the 5′- or 3′-end of oligonucleotides leads to two- or three-way branched derivatives by using 1,4- di- or 1,3,5-triethynylbenzene (**13**), respectively.[76,77] It was demonstrated that a similar assembling strategy of branched architectures is applicable to more complex NA blocks. In these cases, linkers based on aliphatic diamines (*e.g*., ethylenediamine) are used to cross-link two salicylaldehyde residues connected to the oligonucleotide chains (**14**). It was shown that the proximate positions of two salicylaldehyde residues in complementary complexes promotes the conjugation. This strategy for the assembly of DNA structures by means of diamine bridges and aldehyde-containing oligonucleotides (structure **14**) provides diverse linear and branched structures, in particular, those with numerous branches.[76,77]

One more approach to the preparation of branched structures is based on the use of thiol-containing derivatives. For this purpose, a cystamine residue is attached to the terminal or internucleotide phosphate group. The resulting SH-containing NA chains are cross-linked using bismaleimide linker **15**[78] or by forming disulfide bond **16**.[79,80] The assembly of the X-shaped NA block was demonstrated by attachment of four cystamine-containing oligonucleotides to the substituted porphyrin residue **17**.[81,82]

In addition, NA fragments can be linked non-covalently, for example, through interactions involving NA-specific ligand residues present in oligonucleotide derivatives such as oligocarboxamides, high affinity ligands of the DNA minor groove based on *N*-methylpyrrole and *N*-methylimidazole[89,90] or intercalator residues.[91]

Branched oligonucleotide blocks are also assembled through the formation of coordination complexes with metals (see Table 3). It was shown that two modified oligonucleotides can be paired by assembling complexes like $[Ru^{II}(bipy)_2(Imd)_2]^{2+}$ (**18**) (bipy is 2,2′-bipyridine, Imd is imidazole),[83] $[Ru^{II}(bipy)_3]^{2+}$ (**19**)[84] or $[Fe^{II}(tpy)_2]^{2+}$ (**20**) (tpy is 2,2′:6′,2″-terpyridine).[85] In the first and third cases, this is done by adding an imidazole and a terpyridine residue, respectively, on the 5′-end of each oligonucleotide; in the second case, both oligonucleotides are attached to one chelating bipyridine residue. The addition of an appropriate metal cation gives rise to a coordination complex whose structure predetermines the predominant orientation of the brought together oligonucleotide chains relative to each other. For instance, the use of complex **20** as the oligonucleotide joining point results in the predominant orientation of the two NA chains at 90°.[85] In complexes of type **18**, the chains are arranged at ~70°.[83]

The formation of a four-way branched NA block was demonstrated in relation to nickel(II) complex **21** and substituted 1,4,8,11-tetraazacyclotetradecane (tactd).[86] The presence of the metal cation makes the resulting complexes rigid. Apparently, due to repulsion between the NA chains, they point predominantly to the vertices of a tetrahedron.[86] This type of DNA blocks is synthesized using appropriate synthons and chelating groups that bear the required number of hydroxy groups for branching the NA chain.

A coordination complex of type $[Ru^{II}(bipy)_3]^{2+}$ (**22**) with six oligonucleotide arms attached to 4 and 4′ positions of each of the three bipyridine moieties were obtained.[87] In this case, for decreasing the steric effects, the oligonucleotide fragments are introduced through aliphatic C_9 linkers. In this coordination complex, nucleic acids point to vertices

of an octahedron. The formation of intricate branched DNA blocks based on the coordination complexes of cobalt,[92] copper and silver was also described.[93]

Thus, a broad gamut of NA blocks formed using modified oligonucleotide components is presented in the literature. It is clear that combination of several types of modifying agents for oligonucleotide structure opens up the possibility for preparing even more intricate structures. The groups that are introduced into DNA as branching or bending points can function as linkers connecting several DNA blocks and as sites of attachment of additional functionally significant units. The use of chemical modifications in the DNA design would provide the preparation of unique supramolecular blocks with structural features such that are very difficult or impossible to achieve when native oligonucleotides are used.

Thus, the diversity of DNA building blocks needed for DNA architectonics is achieved owing to the natural DNA analogues, *i.e.*, synthetic oligonucleotides that self-assemble through classical types of interaction. For the control of the structure and stability of monomeric blocks, it is very useful to form irregular elements in them such as transition linkers based on single-stranded fragments or hairpin structures. A considerable extension of the range of available shapes and functions of DNA nanostructures can be attained only by using additional structural parts based on other biomolecules (RNA, proteins), non-nucleotide residues (organic compounds, metal complexes and so on). The association of DNA blocks that contain stable branching and/or bending points opens up the way to DNA nanostructures of almost any shape.

III. Static DNA nanostructures

This Section gives a systematic account of the diverse static DNA objects available to date. The attention is focused on the aspects related to the efficiency of association of supramolecular structures depending on the structural rigidity of the constituent blocks and organization principles.

There exist several key methods for the self-assembly of nucleic acids to DNA nanostructures of desired shape and size.

— The simplest 'mix and go' method is based on simultaneous mixing of constituents, each of them containing a sticky end responsible for particular positioning of the formed nanostructure.

— The bottom-up (hierarchical) self-association method includes successive interactions of the components. In this case, the assembly of DNA objects also allows for simultaneous mixing of all oligonucleotide components. However, by strict temperature control during the association process and owing to the considerably differing thermal stability of the presumed helical fragments of the future object, the assembly can be put in order. The step-by-step cooling of the reaction mixture programmes the successive association of system elements up to the final structure.

— In the algorithmic organization, DNA blocks are pre-formed in separate systems, and a unique binding site is introduced into each of the blocks. On stoichiometric mixing under controlled conditions, the desired DNA object is assembled upon the association of components that is pre-determined by unique binding sites. This method is usually applied to the preparation of DNA objects based on a series of prefabricated branched blocks the components of which differ by the sticky end structure.

A special mention is deserved by a relatively new method for the design of DNA nanostructures called DNA origami. In this case, the DNA object is fabricated by the 'mix and go' principle. A specific feature of this method is that one long NA chain serves as the scaffold; it is 'packed' to form the desired structure by means of shorter oligonucleotides functioning as staples.

It is noteworthy that many self-assembling DNA objects were obtained by combination of several approaches.

1. Discrete DNA objects

a. Two-dimensional closed DNA structures

Despite the rigidity of the dsDNA polymer chain, combination of regular double-stranded DNA fragments with structure-perturbing elements may produce closed structures based on native DNA molecules. The presence in dsDNA of specific features such as nucleotide mismatches (especially if there are several of them in succession),[94] internal loops (nucleotides having no complementary base on the opposite strand),[95–97] single-stranded regions,[73,91] oligo(dA)–oligo(dT) tracts[98,99] causes bending of the obtained irregular DNA fragment, thus promoting the formation of a closed ring structure (Fig. 4*a*). The preparation of DNA rings upon closure of 100–200 bp long NA fragments by means of sticky ends was described. The dsDNA rings of a similar length can also be formed from duplexes containing specific sites recognized by DNA-binding proteins (Fig. 4*b*).[53,58,100] The double helix ends can also be connected by implementing high-affinity biotin–streptavidin interaction. Indeed, dsDNA fragments (87–485 bp) bearing two biotin residues on the 5′-ends acquire the ring shape in the presence of streptavidin (Fig. 4*c*).[101]

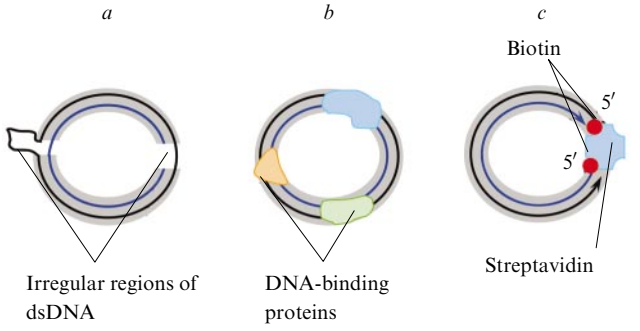


Figure 4. DNA rings based on dsDNA fragments with irregular regions (nucleotide loops and single-stranded regions) (*a*),[73,91,95–97] with regulator protein binding sites (*b*)[53,58,100] and biotinylated at 5′-ends (*c*).[101]

Nano-sized rings can also be produced by chemical ligation of the oligonucleotide components. For example, the unique Cu^{I}-catalyzed [3 + 2]-dipolar cycloaddition of azides to terminal acetylenes (so-called, click reaction) proved to be rather convenient for the preparation of a 72 bp long dsDNA ring. For the formation of the dsDNA

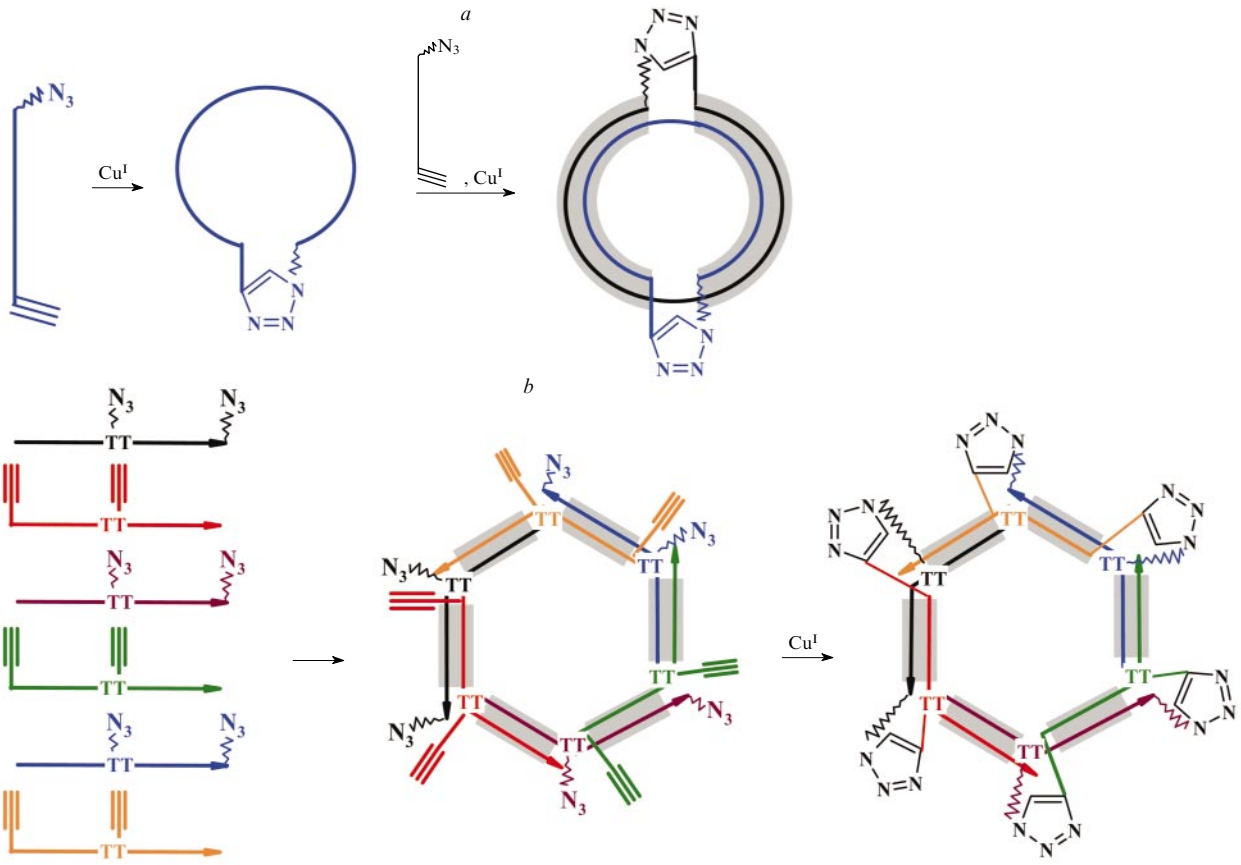


Figure 5. Two-dimensional DNA structures: DNA ring (*a*)[102] and DNA hexagon (*b*)[103] obtained using click-chemistry approaches. For the sake of clarity, some bonds are shown as zigzags.

ring, a closed scaffold oligonucleotide was first obtained by the click reaction between its ends modified by alkyne and azide residues (Fig. 5 *a*).[102] Then a complementary oligonucleotide modified in a similar way was attached to the resulting single-stranded DNA (ssDNA) ring by a ligation process. The obtained DNA rings were fastened by a covalent linker with a 1,2,3-triazole residue as a core between the terminal fragments of the DNA chain.

One more illustrative example of the assembly of two-dimensional DNA structures within the framework of this synthetic approach is the formation of a closed DNA hexagon. For this purpose, a special set of modified oligonucleotides was designed; three of them contained each two alkyne residues (on the 5′-end and in the central part) and the other three contained each two azide residues (on the 3′-end and in the central part) (Fig. 5 *b*).[103] The reactive groups approached each other as the cyclic complex was formed, and the covalent fastening of this complex upon the addition of Cu^{I} gave the desired DNA hexagon. It is noteworthy that the required flexibility of the building block structures was provided by introducing a dithymidylate domain, which remained single-stranded in the final structure, into the central part of each oligonucleotide. These linker domains determined the positions of angles in the DNA hexagon.

In the general case, DNA polygons, *i.e.*, two-dimensional closed structures having bending points in the nanoobject backbone, may be formed by using either only native

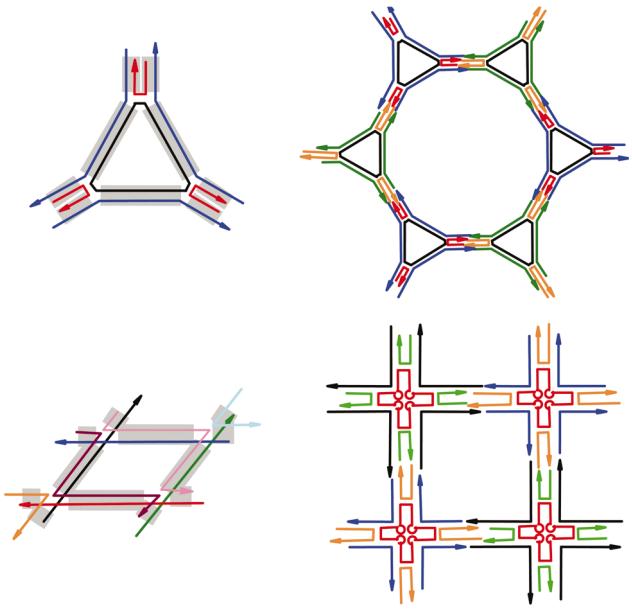


Figure 6. Examples of DNA polygons based on native oligonucleotide components.[104–109]
Here and in Figs 10 and 11 the grey background is omitted in more complex structures for simplicity.

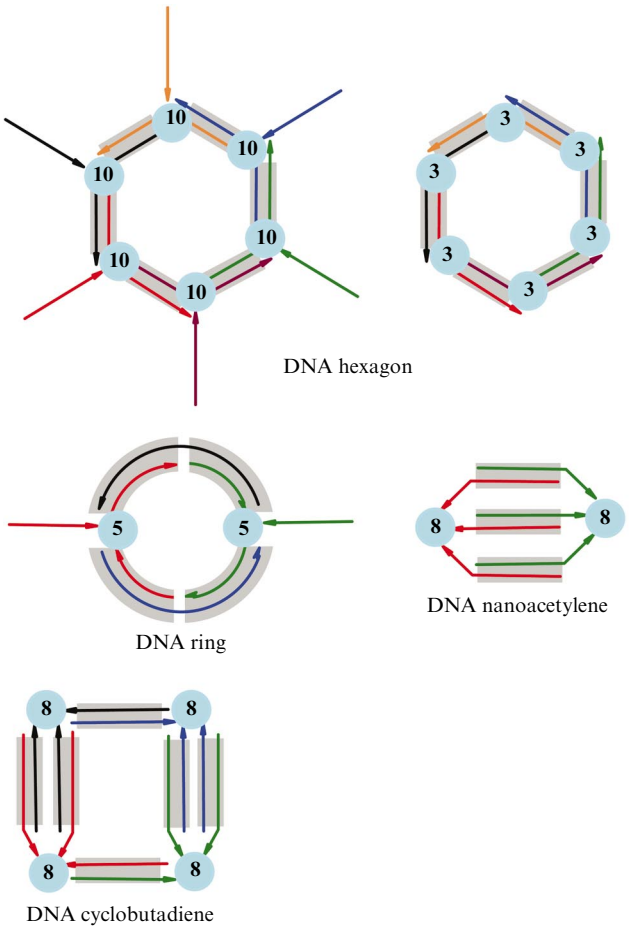


Figure 7. Examples of two-dimensional DNA structures — DNA hexagons, DNA ring, DNA nanoacetylene and DNA cyclobutadiene, which are formed from oligonucleotide components modified by the residues of 1,3,5-tris-(6-hydroxyhexynyl)benzene (**10**),[73] 1,3-bis(4-hydroxyphenyl)benzene (**3**),[63,110] *N*(4)-(6-hydroxyhexyl)-5-methyl-2′-deoxycytosine (**5**)[68] and tris(3-hydroxypropyl)aminomethane (**8**),[71] respectively.

oligonucleotides or their combinations with modified analogues. In most cases, the polygon sides are native dsDNA fragments and the vertices are fabricated from native (Fig. 6) or modified oligonucleotides (Fig. 7). Using A and DA blocks as the branching points, DNA triangles,[104,105] squares[106–109] and other figures were obtained. Typical examples of these DNA structures are shown in Fig. 6. The modified blocks can associate to give tri-, tetra-, penta- and hexagonal two-dimensional objects (see Fig. 7).[63,73,110] Moreover, the nature of the non-nucleotide insertions, for example, in the case of DNA hexagons determines whether the resulting supramolecular structure would be self-sufficient or it would bear single-stranded sequences protruding from the corners and capable of coupling with similar DNA blocks (see Fig. 7).[73] The modified nucleotide units [based on *N*(4)-(6-hydroxyhexyl)-5-methyl-2′-deoxycytosine (**5**), see Table 1] can also be used for increasing the functional complexity of DNA objects. A pair of appropriate branched oligonucleotide derivatives can be complexed to give a ring structure with protruding single-stranded domains (see Fig. 7).[68] A pair of oligonucleotides that are branched by the tris(3-hydroxypropyl)aminomethane (**8**) residue (see Table 1) and bear pairwise complementary oligonucleotide sequences are self-assembled to give a more intricate closed object: nanoacetylene.[71] Four such building blocks form a DNA analogue of cyclobutadiene (see Fig. 7).[71]

Two-dimensional nanostructures based on DNA can act as separate objects in some investigations. However, most often, they are regarded as building blocks for the fabrication of even more complex items: three-dimensional discrete shapes (DNA polyhedra) or two-dimensional shapes with various periodic patterns. The association of two-dimensional structures occurs as a rule through the preliminarily introduced units capable of intermolecular interactions (for example, sticky ends), *i.e.*, single-stranded fragments that are not involved directly in the formation of the DNA block.

The DNA objects of this type are mainly synthesized by the 'mix and go' method. The oligonucleotide components are mixed in a stoichiometric ratio, the reaction mixture is heated to 95 °C and then slowly cooled. Conversely, if DNA rings are assembled using a biotin – streptavidin system, the closed structures are mainly formed on fast cooling to 4 °C.[101]

b. Three-dimensional DNA polyhedra

The building blocks used in the design of three-dimensional DNA polyhedra are the same as used to obtain two-dimensional objects. In particular, three-dimensional objects are most often fabricated using branched A and DA blocks, which can be assembled to give a DNA tetrahedron,[33,111–113] cube,[114,115] octahedron,[116,117] bipyramid,[118] dodecahedron,[33] icosahedron[34,119] and more intricate objects.[33,120] The three-dimensional objects are designed by introducing single-stranded fragments acting as flexible hinges into the building block branching point (Figs 2 and 8 *a*,*b*). The presence of these hinges plays a crucial role for the effective formation of three-dimensional objects.[33,34,112,121] Indeed, no assembly of a DNA tetrahedron occurs on the basis of three-way branched 3A blocks devoid of single-stranded fragments. The presence of even short single-stranded regions (1–2 nucleotides) in the branching point allows the dsDNA edges of the resulting polyhedron to be located at 60°; finally, this determines the

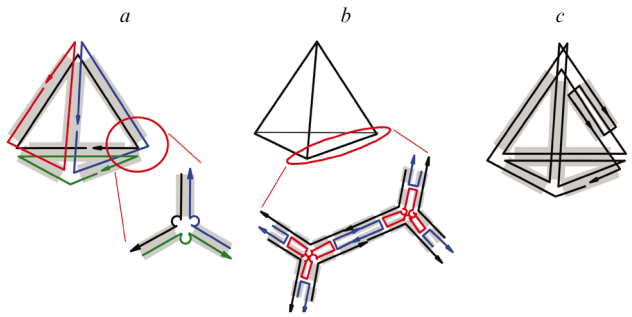


Figure 8. Schematic view of DNA tetrahedra designed based on 3A (*a*)[111,112] and 3DA (*b*)[33] blocks with single-stranded regions at the branching point and also using one 286-mer polynucleotide chain as the structural unit (*c*).[113]

high (up to 95%) efficiency of the assembly of the expected DNA tetrahedron (see Fig. 8 *a*).[111, 112]

He *et al.*[33] demonstrated the preparation of various three-dimensional DNA objects based on one and the same type of 3DA block by varying the size of single-stranded fragments incorporated therein. Using low concentrations (< 100 nmol litre^{-1}) of oligonucleotide components that form 3DA blocks with pentanucleotide single-stranded regions in the structure points, a DNA tetrahedron was obtained in $\sim 90\%$ yield (see Fig. 8 *b*). At higher concentrations of oligonucleotides (500 nmol litre^{-1}), DNA blocks with a trinucleotide linker form supramolecular dodecahedron and fullerene analogues in 76% and 69% yields, respectively. In these cases, short linkers make the edges to be arranged at an obtuse angle, which is characteristic of penta- (108°) and hexagonal faces (120°) of the dodecahedron and fullerene, respectively. A flexible long linker (5 nucleotides) causes a more pronounced bending of the helix, thus favouring the formation of a DNA tetrahedron with triangular faces (60°).[33]

The effect of the local structure and concentration of the monomeric blocks on the resulting shape of the DNA nanoobject was also observed when using the five-way branched 5DA block.[34] The 5DA blocks with single-stranded linkers 5 nucleotide long form mainly DNA icosahedra when present in a concentration of 20 nmol litre^{-1}. Less flexible units having short single-stranded fragments present in higher concentrations form more complex associates, namely, extended periodic lattices.[34] The researchers cited also note that a considerable elongation of single-stranded regions in the nodal points of the DNA blocks and the concomitant increase in the mobility of double-stranded edge fragments would most likely be detrimental for the formation of closed nanoobjects.

For successful design of discrete DNA objects, it is necessary to ensure the optimal rigidity and stability of the desired structure. The crucial role for the formation of DNA polyhedra in the desired right shape is played by the structure of their edges, which are formed most often from native DNA duplexes[112, 114, 118, 119] or more rigid DNA domains, for example, two parallel DNA duplexes connected by crossover points.[33, 34, 115–117] In addition, note that three-dimensional structures with triangular faces (for example, tetrahedron or octahedron) are less prone to change shape and are more stable than the DNA objects whose faces are framed by more edges (dodehedron, *etc.*). This fact was reported in a publication.[33]

Modified components bearing non-nucleotide residues are also utilized for assembling DNA polyhedra. A DNA dodecahedron can be formed upon association of branched oligonucleotides in which branching is formed by the 1,3,5-tris(3-hydroxypropyl)benzene residue (**11**) (see Table 1).[74] Oligonucleotides modified by insertion of 1,3-bis(4-hydroxyphenyl)benzene (**3**) (see Table 1), which induces bending of the dsDNA block, were also used to create DNA prisms with bases of different shape (triangles to hexagons) and truncated prisms (heteroprisms) with different bases, for example, triangular and hexagonal (Fig. 9).[122]

One of the key factors that are detrimental to the efficiency of the assembly of the designed DNA objects is the use of large numbers of different partially complementary oligonucleotides for the formation of a nanofigure. The resulting association of the desired object requires thorough selection of nucleotide sequences of the components and strict provision for their stoichiometric ratio. A decrease in the probability of side complexation and increase in the yields of desired structures is attained by decreasing the number of oligonucleotide components. This principle was demonstrated in works by Mao, who used symmetric blocks like 3DA, 4DA and 5DA to obtain a number of DNA objects such as DNA tetrahedron (see Fig. 8 *b*),[33] octahedron,[117] dodecahedron,[33] icosahedron [34] and so on.

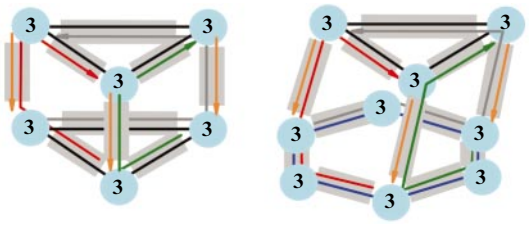


Figure 9. DNA prisms based on oligonucleotide components modified by the 1,3-bis(4-hydroxyphenyl)benzene residue (**3**).[122]

The problem of maintaining the stoichiometry of the oligonucleotide components is minimized when DNA objects are based on one extended NA fragment with a specially selected sequence. Using 286-mer ssDNA that tends to undergo intramolecular structuring, it is possible to form a DNA tetrahedron in 90% yield (at a ssDNA concentration of 50 nmol litre^{-1}) (see Fig. 8 *c*).[113] It is noteworthy that NA fragments, precursors of this structure, can be produced using genetic engineering or molecular biology approaches.

One more factor determining the formation efficiency of three-dimensional DNA structures is the selected association pattern of the constituents. In the pioneering works, the polyhedra were obtained using the 'mix and go' approach. The formation of complex supramolecular structures in solution without the isolation of by-products (undesired associates) decreased the yield of the final structure to 10%.[114] The development of approaches based on solid-state assembly of DNA structures allowed the isolation of intermediate building blocks. This strategy was used to prepare a truncated DNA octahedron,[123] which was assembled in several stages. First, oligonucleotide components were assembled to form DNA squares, which were then covalently locked by ligation and attached to a support. Then the DNA object was synthesized on the solid phase by the algorithmic procedure, and the desired DNA structure was isolated (Fig. 10). The vertices of the DNA squares are formed in this system by four-way branched 4A type blocks where a pair of DNA branches serves for the formation of the square edges and the remaining two double-stranded hairpin structures contain specific restriction sites. By action of restriction endonucleases, the hairpin domains are split to give sticky ends, which are used either for the temporary attachment of the building block to the solid support or for positioning of the prepared block in the structure being designed. Thus, hairpin elements function as specific protecting groups that are removed by restriction endonucleases. By using this type of algorithmic strategy for the synthesis of DNA polyhedra,

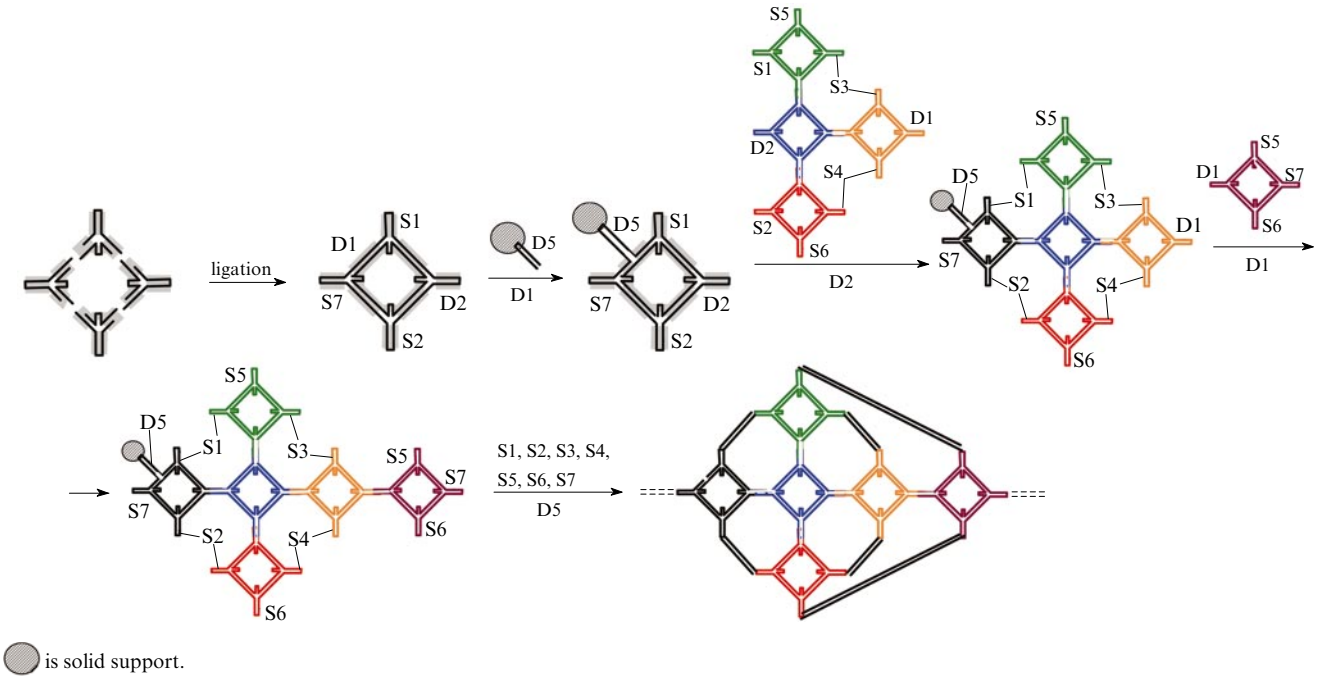


Figure 10. Design of truncated DNA octahedron on the solid phase based on 4A motifs.[123] Restriction endonuclease cleavage sites are indicated: S1 – S7, D1, D2, D5.

product yields of 50% in each synthetic stage were achieved.[123]

The design of DNA polyhedra using branched blocks pre-assembled in independent systems (algorithmic method) was also demonstrated for the formation of objects in solutions. This approach proved useful in the formation of a DNA icosahedron (Fig. 11).[119]

Yet another effective expedient that provides high yields of the desired DNA polyhedron is so-called hierarchical assembly.[111, 112, 124] According to Goodman,[112] this strategy leads to a high yield of a DNA tetrahedron (95%) whose

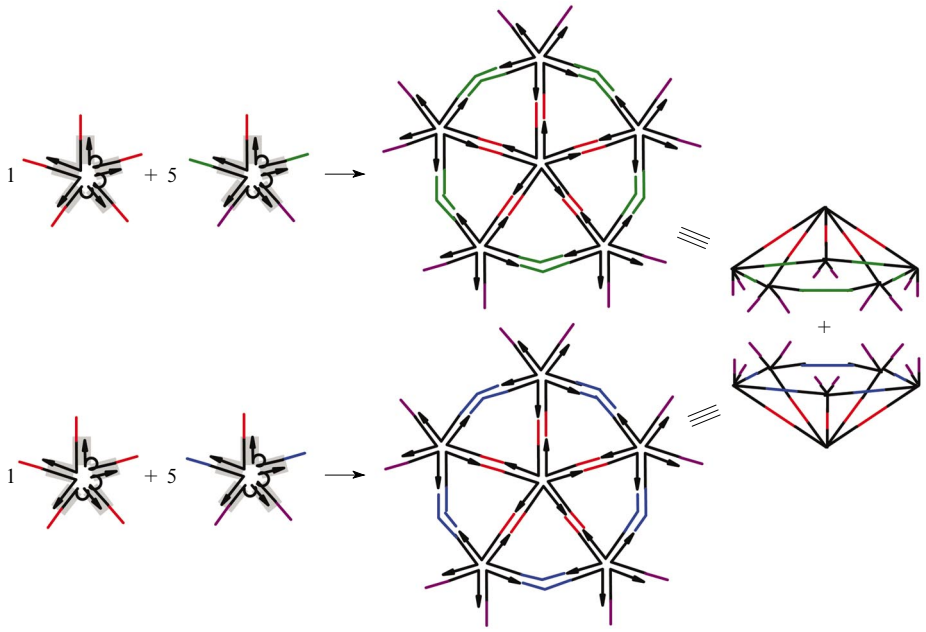


Figure 11. Assembly of DNA icosahedron based on monomeric branched 5A DNA blocks prefabricated in independent systems.[119]

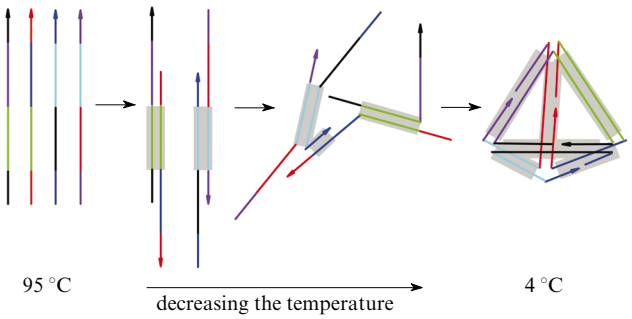


Figure 12. Assembly of DNA tetrahedron by hierarchical association of constituent oligonucleotide components.[7, 112]

helical constituent regions have different 'melting points' (Fig. 12). Apart from the DNA tetrahedron, a DNA octahedron was designed in a similar way by slow (0.25 °C min^{-1}) cooling from 65 to 16 °C of an equimolar mixture of eight oligonucleotides consisting of three helical regions with different stability.[124]

Thus, literature presents a wide diversity of discrete three-dimensional DNA structures involving native or modified oligo- and polynucleotide components. Successful assembly of three-dimensional DNA objects is based on the selection of monomer blocks and the pattern of their self-assembly. In the formation of DNA blocks, a significant issue is the design of branching points. The use of the same blocks that differ only by the branching point structure gives rise to objects of a variety of shapes.

2. Periodic DNA patterns

Periodic structures based on DNA are formed upon successive binding of the same or several different DNA blocks. The blocks can be connected to form one-, two- and three-dimensional periodic patterns; two-dimensional lattices can, in turn, roll up thus forming so-called DNA nanotubes. Characteristic features of organization of this structures are discussed in detail below.

a. DNA lattices

The first works on the design of periodic two-dimensional structures are based on the use of rigid building blocks, namely, double [125–129] or triple [130] crossovers (DX or TX) linked through sticky ends. A key structural feature of these blocks that promotes their association to give extended two-dimensional patterns is the appropriate length of the duplex fragments in the scaffold blocks that form the structure and determine the position of crossover points (Fig. 13 *a*). Most often, this is controlled by the number of half-turns of the DNA helix between the neighbouring crossover points. The formation of a planar macromolecular structure is facilitated by an odd number of half-turns between the branching points; this orients the adjacent duplexes coupled by crossovers in such a way that the principal axes of their helices fall in the same plane. The size of the periodic DNA plates based on DX and TX blocks can reach several hundreds of nanometres along and across, the average size of the monomeric block being $\sim 4 \times 15$ nm. The use of symmetric DX blocks formed by only two different oligonucleotides resulted also in more extended structures ($\sim 30 \times 1$ μm).[128]

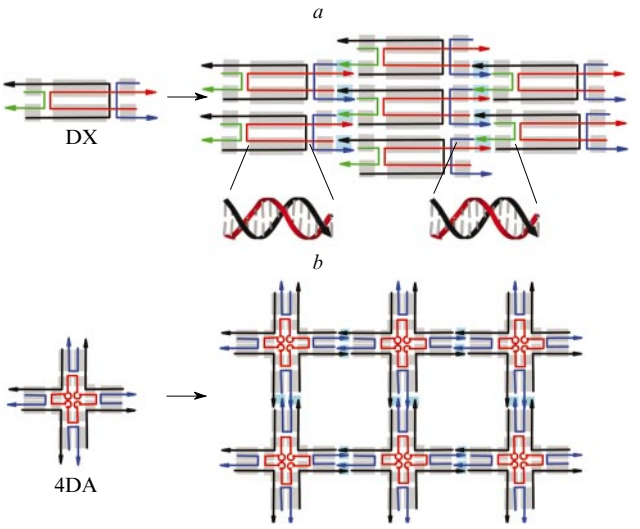


Figure 13. Schematic view of periodic two-dimensional DNA patterns based on DX (*a*) [125–129] and 4DA (*b*) DNA blocks.[28, 31, 32, 109, 132]

Periodic DNA patterns were also formed using rigid DNA blocks of the DA type. The assembly of these blocks affords DNA lattices with pores shaped like regular polygons. The pore shape is determined by the steric and/or energy benefit of positioning of the rigid edges. The DNA lattices thus obtained had pores shaped like regular polygons: triangles,[105, 131] squares (Fig. 13 *b*) [28, 31, 32, 109, 132] and hexagons.[105, 133–135] The assembly of type 5DA blocks affords lattices with irregular pores, *i.e.*, the same lattice can have both triangular and square pores.[34]

The use of rigid 3DA blocks results in the formation of DNA plates comparable in size with patterns based on DX and TX blocks. The size of DNA pattern can reach hundreds of nanometres and in some cases, one of the parameters of the lattice formed can be tens of micrometres. By using symmetric DA blocks that require fewer different oligonucleotide components, the size of DNA patterns can be increased to 1 mm.[32, 105]

Periodic DNA structures can also be constructed based on rather flexible branched A-type blocks.[34, 107, 108, 136] The preparation strategy of DNA objects from these blocks is based on the so-called tensegrity principle (the word is derived from 'tension' and 'integrity').[136] The key of this strategy is the fact that combining flexible blocks into a complex multicomponent structure diminishes the degrees of freedom of the constituents, resulting in the formation of the target DNA object having the desired rigidity. The self-assembly of flexible blocks affords stable DNA lattices with, for example, rhombic cells.[107, 108]

Topologically, DNA blocks of DX and TX, A and DA types are planar structures; hence, their assembly gives predominantly two-dimensional patterns. For fabrication of three-dimensional structures, it was proposed to use TXT blocks (see Fig. 2), which represent an assembly of three parallel dsDNA fragments arranged in a prismatic pattern with triangular bases (Fig. 14).[30] In the TXT block structure, the sticky ends are located in different planes; this apparently opens up the possibilities for connecting the

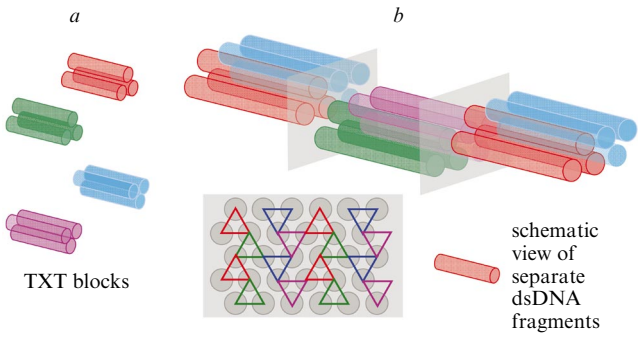


Figure 14. Schematic view of TXT blocks (*a*) and three-dimensional DNA patterns obtained from these blocks (*b*).[30]

blocks with one another to a three-dimensional periodic structure, which is schematically shown in Fig. 14.

By analogy with the principles of design of DNA polyhedra, single-stranded fragments are inserted at the branching points of some constituents to impart the required flexibility to the rigid blocks. The linker fragments prevent the stacking interactions between the bases of the DNA arms and relieve the strain of the periodic lattice, thus providing the optimal arrangement of the cell edges.[31, 109, 131–135] As in the case of discrete objects, the efficiency of formation of regular DNA-based lattices directly depends on the length of the single-stranded region. Regular hexagonal lattices are formed only if the three-way branched 3DA structures bearing single-stranded regions of 2 or 3 nucleotides in the central part are used as building blocks. The presence of longer (4 nucleotide) or shorter single-stranded regions or their absence results in the formation of small irregular associates whose cells can be square, pentagonal or hexagonal.[134]

On the basis of DNA blocks formed using native oligonucleotides, periodic DNA patterns with limited linear dimensions were obtained. This problem was solved with the EX blocks composed of eight DNA duplexes located in one plane and coupled in pairs by double crossovers.[28] Limitation of the object size by restricting its growth was implemented by selecting the structures of the sticky ends in the EX blocks. The design of the blocks determines the possibility of specific interaction between 25 tiles (17×14 nm) to give a 110×110 nm DNA platform.

It was shown that with two different building blocks based on double crossovers (DAE and DAE + 2J), it is possible to encode information by binary codes using DNA structures.[126] These planar blocks can be distinguishable according to their shape by atomic force microscopy: DAE is a rectangle and DAE + 2J has additional DNA loops protruding from the structure plane. According to the authors, if DAE is taken to be zero and DAE + 2J is taken to be unity, they can be connected to a chain-like structure to give a DNA plate whose shape encodes 5 bits of information: 01101 and the inverted code 10010 (Fig. 15). Furthermore, the information plates can be associated with one another to give a structure with greater information capacity.[126]

Yet another curious aspect of the design of DNA lattices deserves attention. It was shown that objects of different

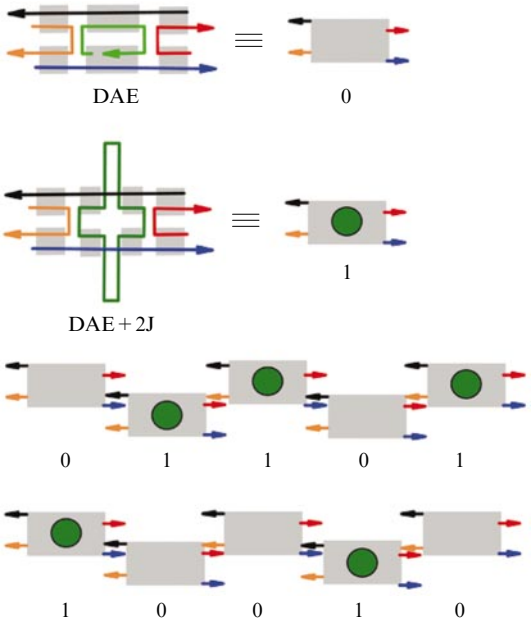


Figure 15. Encoding principle of 5 bits of information (01101 and 10010) by two-dimensional DNA patterns based on DAE and DAE + 2J blocks.[126]

shape can be obtained from one type of blocks by varying the combination of sticky ends and self-assembly conditions. Using DNA parallelograms (Fig. 16), one- or two-dimensional lattices were obtained by introducing sticky ends into DNA fragments that go outwards from the parallelogram corners.[107, 108] The absence of sticky ends on

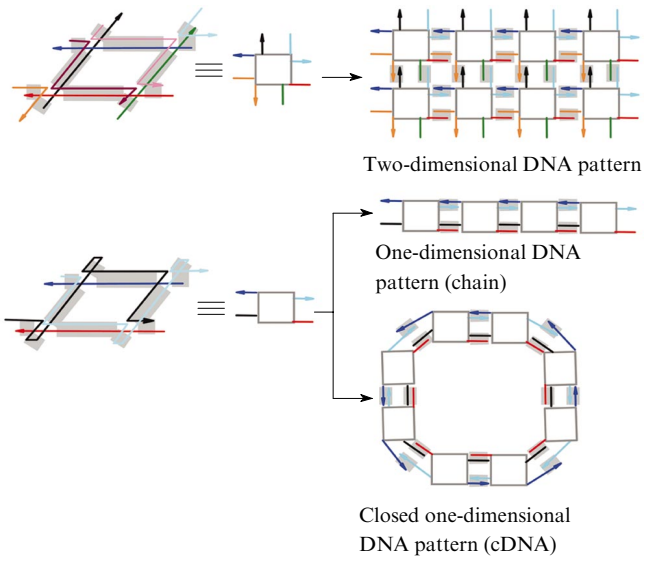


Figure 16. Principles of the change in the direction of assembly of two-dimensional DNA objects based on one building block: DNA parallelogram.[108]

one or several sides of the parallelogram programmably prevents the growth of the supramolecular structure in these directions. When blocks with two types of sticky ends differing only by one nucleotide are used, slow cooling of the system (0.01 °C s^{-1}) increases the fraction of closed (ring) one-dimensional structures (cDNA structures) to 69%, whereas the assembly of similar DNA blocks with one type of sticky ends gives only a 2% fraction of cDNA structures. A decrease in the cooling rate (0.0003 °C s^{-1}) additionally increases the fraction of ring structures in the former system (up to 89%), the circle length increasing in parallel. Fast (3 °C s^{-1}) cooling of both systems results in the predominant formation of one-dimensional chain-like DNA structures.[108]

Periodic DNA structures with larger area can also be assembled using only one specially selected oligonucleotide, for example 32-mer [137] and 52-mer [138] oligonucleotides that contain each four palindromic fragments comprising 6, 10, 10, 6 and 10, 16, 16, 10 nucleotides, respectively (Fig. 17). The production process of multicomponent associates involving these oligonucleotides can be split into the following stages:

1) all possible secondary structures of the monomeric oligonucleotide are denatured at high temperatures;

2) slow cooling accompanied by hybridization of oligonucleotides to an extended concatemer duplex in which protruding single-stranded fragments comprising 6 or 10 nucleotides for 32- or 52-mer oligonucleotide, respectively, are located every 10(16) bp;

3) further decrease in temperature induces interaction of sticky ends, thus ensuring the formation of a two-dimensional crystal (see Fig. 17).

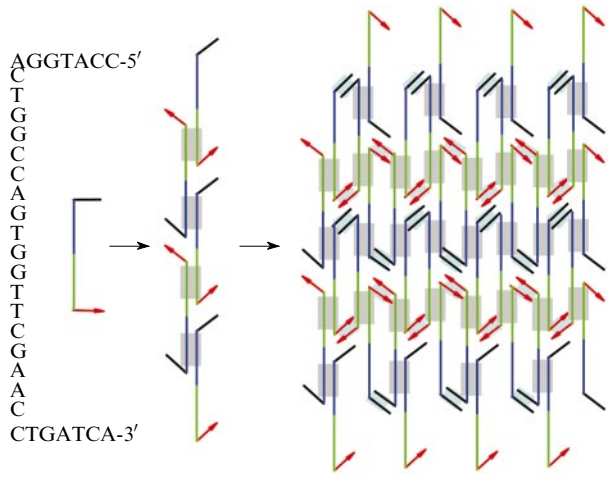


Figure 17. Two-dimensional periodic DNA pattern formed upon the use of one oligonucleotide with four palindromic regions as the building unit.[137]

The final DNA structure is held by double crossovers that are similar to DX blocks containing single-stranded breaks (nicks) in the branching points. This feature is responsible for the flexibility of the obtained DNA lattice, which may roll up to form a tube.[137, 138] The use of only one component in the design of nanoobjects minimizes the problem of observing the specified stoichiometric ratio of the components, which is faced by the design of structures based on multicomponent systems.

Periodic DNA lattices were also designed from modified oligonucleotides. The formation of one-dimensional periodic rail-like structure, *i.e.*, a pair of parallel DNA helices cross-linked by non-nucleotide linker **15** was described (see Table 2).[78] This DNA ribbon is formed according to the concatemer formation principle, the role of the longitudinal staple is played by the native oligonucleotide, while the lateral staple is the bis-oligonucleotide conjugate (Fig. 18 *a*). Association of even relatively short oligonucleotide components (10 nucleotides) in this system furnishes a several micrometre long DNA structure. It is clear that upon introduction of minor changes into the structures of components, two-dimensional DNA lattices can also be formed.

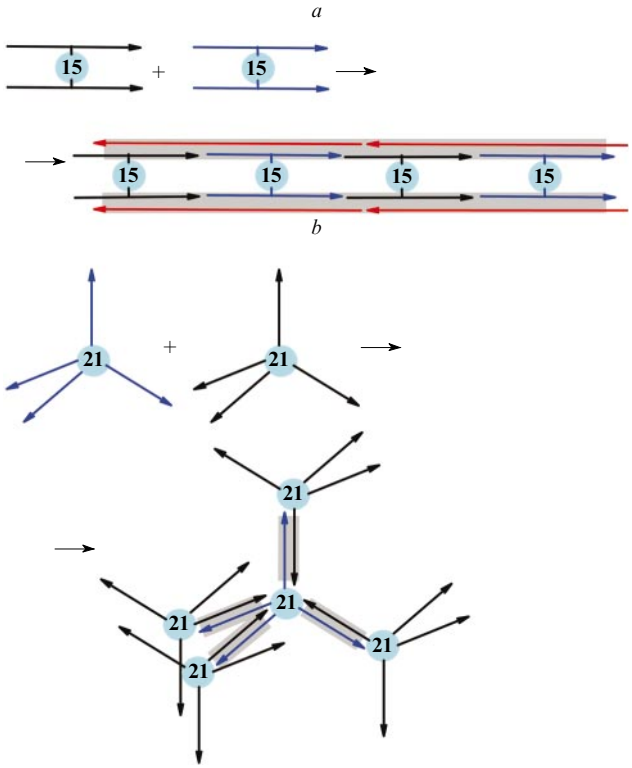


Figure 18. Principles of construction of periodic DNA patterns: one-dimensional DNA pattern based on dsDNA cross-linked by bis-maleimide linker **15** (*a*) [78] and three-dimensional DNA pattern formed using modified oligonucleotides based on nickel(II) complex with 1,4,8,11-tetraazacyclotetradecane **21** (*b*).[139]

The prospects of constructing three-dimensional DNA patterns based on modified oligonucleotide architectures were studied in relation to a complementary pair of branched conjugates combining each four identical 20-mer nucleotide chains that go from the nickel(II) complex with 1,4,8,11-tetraazacyclotetradecane **21** (see Table 3, Fig. 18 *b*).[139] The complexation of each chain within the conjugate is hampered by the electrostatic repulsion of the large number of DNA fragments concentrated around the

converging structures. The effect of repulsion can be overcome by increasing the ion strength of the solution. Indeed, in a solution containing 100 mmol litre^{-1} of $MgCl_2$ and 50 mmol litre^{-1} of NaCl, branched molecules with one type of DNA arms interact almost completely with similar complementary DNA blocks and form pentamers. A stoichiometric mixing of this pair of branched oligonucleotide structures affords mainly high-molecular-mass associates. The obtained results support the assumption that modified blocks of this type are potentially applicable for the assembly of three-dimensional DNA lattices.

In the DNA architectonics, a successful attempt was made to obtain three-dimensional DNA patterns with a cubic arrangement of unit cells.[140] These cells comprise 24 short duplexes (6 bp) and 96 Ni^{2+} ions. The three-dimensional pattern is held by cross coordination interactions involving the metal cations, guanine N(7) atoms and phosphate residues. The DNA duplexes are parallel to the four diagonals of the cube, thus creating an inner cavity in the cell with a ~50 Å diameter shaped like a curved icosahedron. The construction of three-dimensional periodic DNA crystals was also reported in other works.[141, 142]

Three-dimensional DNA patterns were assembled based on the biotin–streptavidin interactions.[143]

It is necessary to emphasize that the most successful assembly of DNA multiassociates from any of the above-described blocks is often attained by using the algorithmic strategy. The separation of the key stages of 'synthesis' of the desired DNA structure, *i.e.*, preparation of the blocks and the assembly, allows one to carry out the stages under the optimal, specially selected conditions.

Thus, the extended periodic DNA patterns are designed from the same blocks as the discrete DNA structures, in particular, branched blocks prepared using either native or modified oligonucleotide components. The shape of the DNA blocks and the arrangement of sticky ends through which the blocks are assembled into a complex structure predetermine the formation of one- or two-dimensional DNA patterns. It was demonstrated that three-dimensional periodic structures made of DNA molecules can in principle be obtained, although the formation of such surfaces is hampered by the electrostatic repulsion and by steric hindrance between the dsDNA fragments approaching each other.

b. DNA nanotubes

The DNA nanotubes based on synthetic DNA are most often produced upon closure two-dimensional periodic DNA patterns. The DNA nanotubes described in the literature are usually designed using the same building blocks as used to obtain periodic objects, namely, double (DX)[81, 144–147] and triple (TX)[148, 149] crossovers, 4DA blocks,[31] and also more complex FX, EX and TwX blocks, which are assemblies of 4, 8 and 12 parallel dsDNA fragments combined by double crossovers.[29] From the energy standpoint, closure of a two-dimensional DNA lattice to form a tube is favourable as this decreases the system free energy due to the formation of duplexes through the edge sticky ends, which remain free in a planar DNA object. However, the proximity of the edge constituents of the multiassociate is possible only if the planar structure rolls up, which is programmed by introducing structural motifs that induce bending strain into its building blocks. A most common approach to the control of bending of crossover-joined planar blocks is selection of the length of the dsDNA region between the crossovers. In this case, the key factor is the number of helix half-turns between the sites of NA sequence transition from one structural element to the other within the same block or in the linker regions between the neighbouring blocks. Unlike planar lattices, nanotubes are efficiently formed when DX crossovers with even numbers of half-turns of dsDNA fragments between the junctions are used.[31, 145] For example, DNA nanotubes can be designed from DAE-E, which is a double (D) antiparallel (A) crossover with an even number of half-turns of the DNA helix (4 half-turns) between the branchings of one tile (E) and two neighbouring tiles (-E) (Fig. 19 *a*). The inner angle of these blocks is ~153°,[145] which ensures the closure of the DNA structure.

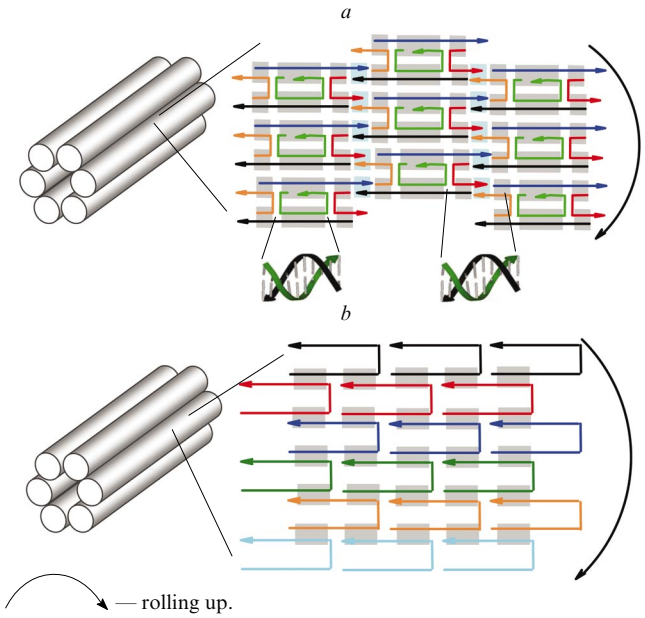


Figure 19. DNA nanotubes based on DAE-E block (*a*)[145] and a system of complementary oligonucleotides (*b*).[150]

The DNA nanotubes with hexagonal symmetry relative to the principal axis were also prepared using specific DNA blocks that combine six parallel DNA duplex fragments interlaced by crossovers. Closure of the structure based on this block is facilitated by the crossover sites being located every 14 bp, which specifies a 60° shift of the principal axis of every third DNA duplex relative to the axes of the two preceding ones.[146, 149]

Simpler systems for the construction of DNA nanotubes based on a single oligonucleotide component were also reported. Special 32- mer[137] and 52-mer[138] oligonucleotides whose sequences comprised four palindromic segments were designed (see the DNA lattices in Fig. 17). Each of these oligonucleotides served to form two-dimensional lattices.[137, 138] The DNA plates are sufficiently flexible to roll up to form tubes. The efficiency of this process can be controlled by selecting the concentration of the oligonucleotide component. Since closure of DNA tubes is an 'intramolecular process', it would apparently be preferred at low oligonucleotide concentrations (1 μmol litre^{-1}).

A specific system of oligonucleotides has been proposed for the design of DNA nanotubes. Each oligonucleotide laid according to a horseshoe pattern can form duplex fragments with the appropriate parts of four adjacent oligonucleotides.[150] Thus, each oligonucleotide acts as a linker and simultaneously as a part of the reinforcing frame of the nanostructure (Fig. 19 *b*). The DNA plate obtained in this system has complementary sticky ends on the opposite sides, which are responsible for closure of the structure upon interaction. The diameter of the obtained nanotubes is specified by the number of different oligonucleotides that form the nanotube precursor, *i.e.*, the DNA ribbon of the desired width. The length of the DNA tube is determined, first of all, by the stability of the duplex fragments contained therein, *i.e.*, by the factors similar to the factors that govern the formation of concatemer structures.[150]

The use of additional modified elements as parts of building blocks may be favourable for rolling up of the two-dimensional DNA structures to give nanotubes and for their subsequent fastening. The conjugates of porphyrin residue with four oligonucleotides (**17**) (see Table 2, Fig. 20 *a*)[81] and disulfide bridges (**16**) (see Table 2,

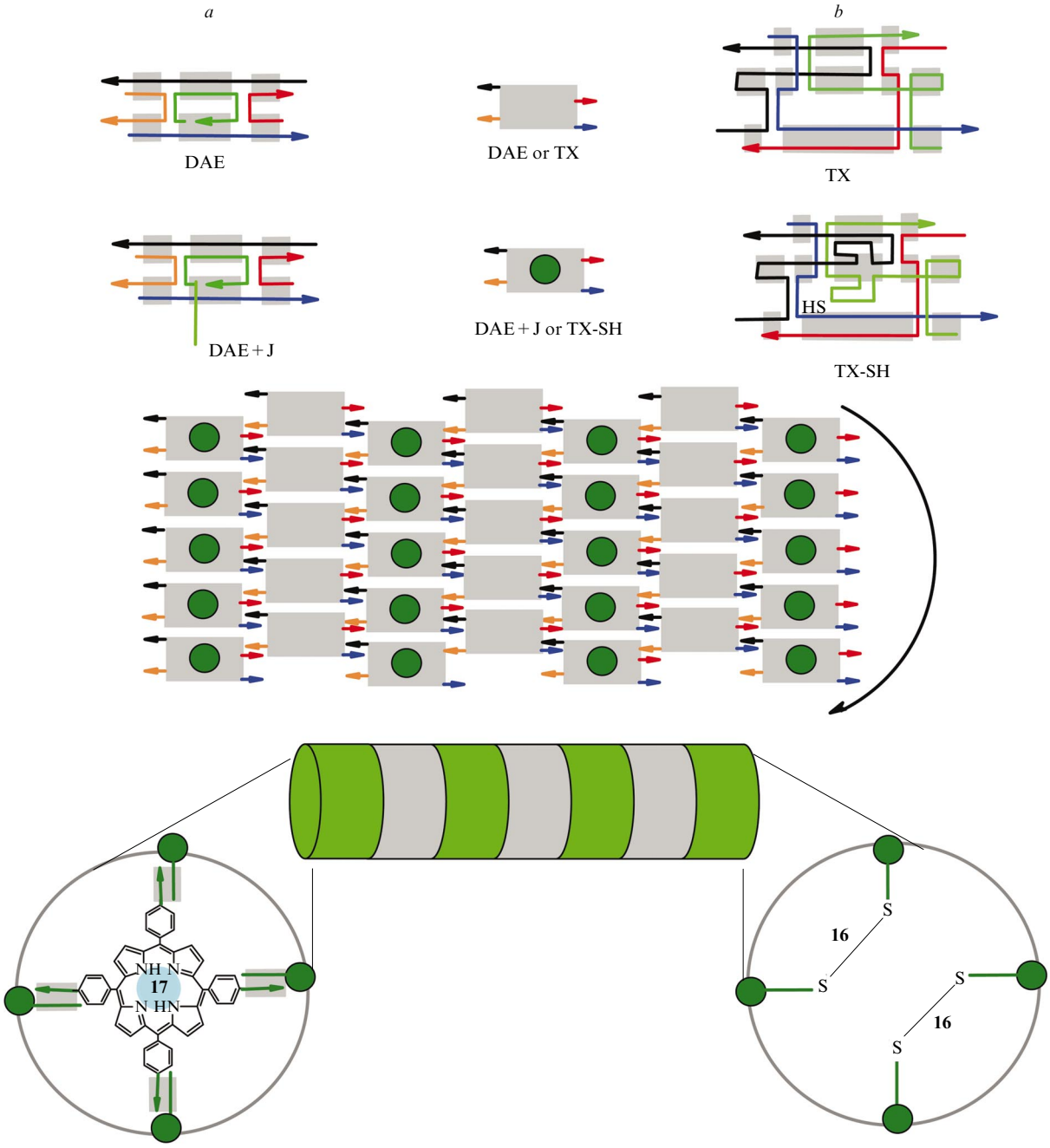


Figure 20. DNA nanotubes using modified elements for securing the conformation: a linker based on porphyrin **17** (*a*)[81] and disulfide bridges **16** (*b*).[148]

Fig. 20 *b*) were used to fasten the structure.[148] In these works, the DNA nanotube walls were assembled from two types of DAE blocks: native and modified ones. In the former case, the structure was modified by additional single-stranded regions protruding from the plane of the block,[81] while in the latter case, two NA loops with thiol groups on the 3′- and 5′-ends were used.[148] The presence of additional sticky ends in the inner cavity of the formed tube allows for the lateral fastening of the structure through four-point attachment of the porphyrin-containing conjugate of four oligonucleotides complementary to the sequences protruding from the DNA nanotube (see Fig. 20 *a*). In this case, the conjugate not only fastens the final structure but also promotes rolling up by successively fixing the DNA lattices around the porphyrin core.[81] The authors noted that the formation of nanotubes is also detected in the absence of the clipping conjugate; however, in this case, extended lattices are mainly formed in the system. The presence of the clipping unit in a concentration equal to one-fourth of the building block concentration induces the quantitative rolling up of the DNA lattices. Association of thiol-containing blocks with their native analogues gives rise to a tube in which the modified residues are directed toward the inner cavity and closely spaced, and the formation of disulfide bonds between them fastens the resulting DNA structure (see Fig. 20 *b*).[148]

It is noteworthy that DNA associates can be fastened by covalent bonds. It was shown[147] that the DNA nanotubes can be ligated by T4 DNA ligase. Fastening of the unusual shape of the DNA structure makes it more suitable for the subsequent handling, in particular, more stable against variations of the environmental conditions: temperature, salt concentrations, electrostatic effects, *etc*.

Note that the fabrication procedure of DNA nanotubes is similar to the preparation of extended lattices. According to instrumental data, the length of the DNA tubes may range from hundreds of nanometres to several tens of micrometres and the diameter is about tens of nanometres. It is clear that the nanotube dimensions depend on the structure of DNA blocks. More flexible building blocks usually afford narrower DNA tubes.[29]

Thus, extended or closed periodic DNA structures (DNA patterns or nanotubes) are obtained from building blocks of the same type. The formation of DNA nanotubes is due to closure of DNA plates. The structures of monomer blocks differ mainly by the number of half-turns of dsDNA regions between the branching points: this number should be odd when extended DNA patterns are to be produced or it should be even for effective formation of DNA nanotubes.

3. DNA origami

a. General characteristics of the method

Several years ago Rothemund[151] proposed a unique approach to the design of DNA structures that differed crucially from the above-described methods of the fabrication of nanoobjects. The DNA origami approach is based on laying of a long (several thousand nucleotides) single-stranded NA scaffold into an object of a specified shape by means of numerous relatively short oligonucleotides (usually $\leqslant 25$ nucleotides) functioning as sequence-specific staples. Each of these oligonucleotides is complementary to several regions of the scaffold remote from one another. By interacting with the corresponding fragments, the oligonucleotide connects them to one another (Fig. 21). Using a prototype of this approach, Shih *et al.*[116] designed a DNA octahedron with the structure based on a long ssDNA (1699 nucleotides) complexed with five 40-mer oligonucleotides. The size of this structure was about 22 nm.

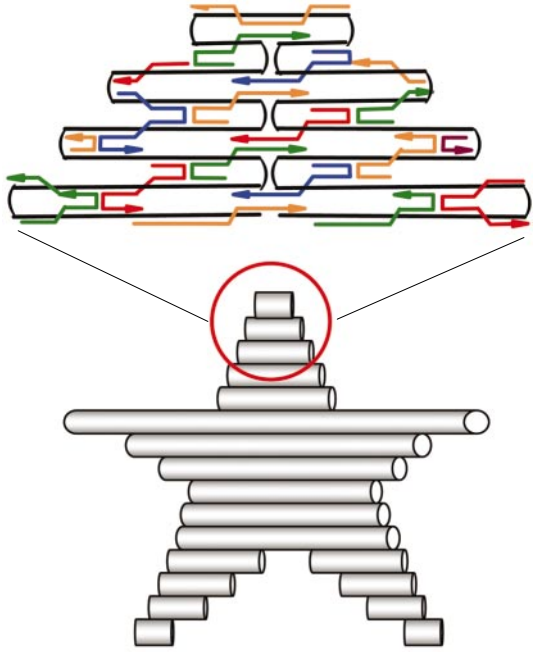


Figure 21. Principle of the DNA origami approach for the design of DNA objects.[151]

The origami type formation of a nanoobject takes place after mixing of all nucleotide components in one reaction mixture upon the simple denaturation – renaturation procedure. However, this process is preceded by multistage computer simulation of system components. First, the desired shape of the nanoobject is selected. Within the object outlines, a long single-stranded NA chain is laid in parallel rows. Then the oligonucleotide staples are designed; this is the most labour-consuming stage of the design. The oligonucleotide structure is selected in such a way that they become complementary to several regions of the scaffold NA sequence that are remote in the primary structure but proximate in the nanoobject to be formed. Thus, the staples form complexes with several sites of the scaffold thus giving crossovers on passing from one site to another. Finally, the specified shape becomes filled with DNA duplexes arranged in parallel and stapled by crossovers, which impart the required rigidity to the structure (see Fig. 21). Crossovers can be separated by any odd number of half-turns of the DNA helix; they turn back the oligonucleotide staples. Evidently, more closely placed crossovers afford more rigid DNA structure. Furthermore, for reducing the adverse influence of electrostatic repulsion arising between parallel DNA chains, the dsDNA length between two crossovers is specified; this controls the gap width between the neighbouring DNA duplexes.[151]

The DNA origami was tested in the design of various two-dimensional DNA objects of size 100×100 nm.[151, 152] A five-point star (see Fig. 21), a smiling face, a dolphin and

other structures were made using the same bacteriophage M13 DNA genome (~7000 nucleotides) as the scaffold. The sets of oligonucleotide staples were specially selected in each case. For each structure, about 200 oligonucleotides with a length of 30 to 70 monomers were used.[151, 152] Subsequently, this procedure was employed to obtain three-dimensional structures [153, 154] and 410 nm-long DNA nanotubes,[155] also starting from the bacteriophage M13 DNA genome.

As opposed to the traditionally used approaches to the assembly of DNA structures, Rothemund's method does not require the maintenance of a definite stoichiometric ratio of the oligonucleotide components. Short oligonucleotides that have not been purified after the synthesis are added to the reaction mixture in a large excess. The assembly occurs fast and gives products in high yields.

The necessity of using a long single-stranded DNA fragment somewhat restricts the scope of applicability of DNA origami. Högberg *et al.*[156] proposed a strategy of using double-stranded DNA scaffold with a length of ~7500 bp. In this case, dsDNA is first denatured under specially selected conditions, while the oligonucleotide staples have been already added to the system. The denaturation is induced by joint action of heating and denaturing agents, for example, formamide; an increase in the formamide concentration in a solution by 1% decreases the dsDNA 'melting' temperature by almost 0.6 degrees.[157] The DNA objects are prepared by heating the reaction mixture of dsDNA and oligonucleotide staples to 80 °C in the presence of 40% formamide followed by fast cooling to 25 °C and by dialysis for the removal of formamide. Converting the effect of the denaturing agent concentration to the temperature equivalent implies that this procedure corresponds to heating of the sample to 106 °C followed by fast cooling to 51 °C and then by gradual cooling to 25 °C.[156] The outcome of these operations is that up to 75% of the extended chain initially participating in the formation of dsDNA fragment becomes involved in the formation of the programmed DNA object. Similar techniques were utilized for efficient design of DNA objects with preliminary denaturation stage at room temperature.[158] All of the constituents of the future DNA structure are mixed in a solution containing a high concentration of formamide or urea. Then, while performing dialysis, the content of the denaturing agent was gradually decreased to 1%, which corresponds to cooling of the reaction mixture and promotes the formation of DNA complexes and finally the formation of the desired DNA structures. The developed method is suitable for the fabrication of DNA nanostructures based on oligonucleotide components bearing, in particular, temperature-sensitive modifications and functional groups.

The origami technique can be used to fabricate DNA objects of diversified shapes. Certainly, this technique is labour-consuming, because a large body of simulation and the synthesis of a broad range of oligonucleotide staples are required. However, the method also has considerable advantages. This principle of design of DNA structures opens up opportunities for the fabrication of highly functionalized multicomponent nanoobjects, as each of the oligonucleotides used can be modified by a particular group. This multifunctional character cannot be achieved in the assembly of periodic DNA structures from a limited set of repeating blocks.

b. Three-dimensional DNA containers prepared by the DNA origami principle

The particular interest in the development of the origami technique is related to the possibility of preparing three-dimensional DNA polyhedra containing an inner cavity. A distinction of these structures from the structures described above is the fact that their faces are polygons filled by dsDNA. Hence, the inner space of these DNA objects is protected on all sides by DNA walls. Using the DNA origami technique, DNA tetrahedron,[159] DNA cube (Fig. 22) [160, 161] and DNA flask were fabricated.[162]

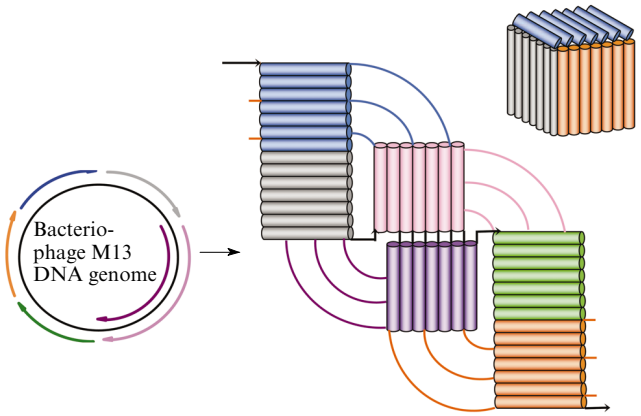


Figure 22. Design of three-dimensional DNA box using the DNA origami technique.[160]

Three-dimensional objects are designed using the advanced origami technique. The key topological difference between two- and three-dimensional objects is that three-dimensional structures have no in-plane boundaries, which is taken into account in the arrangement of the scaffold sequence in the desired shape. In the case of two-dimensional objects, the scaffold sequence is arranged in parallel rows from top to bottom, fills the whole shape and turns back every time as the structure edge is reached. In the case of design of three-dimensional objects, the scaffold sequence is also arranged in parallel rows over the shape surface, thus wrapping the whole inner space.[159] In some cases, the scaffold is represented by ring ssDNA in which some regions are involved in the formation of several faces, each being prepared by analogy with the preparation of planar DNA figures.[160, 161] Linker sequences having no complementary sites in the scaffold sequence are introduced into the oligonucleotides that staple the regions located at the edges of two faces in the final structure. These short linkers (2–4 nucleotides) ensure the possibility of adjusting the faces to one another in the final object.[159, 161]

Three-dimensional objects are usually fabricated in two stages: first, the scaffold is assembled with the majority of short oligonucleotides to give an intermediate structure (a planar or open three-dimensional structure), which is then closed to give a DNA polyhedron upon the addition of more oligonucleotide staples to the reaction mixture. The dynamic radius of the three-dimensional objects thus formed is 40–45 nm, and the volume of the inner cavity is

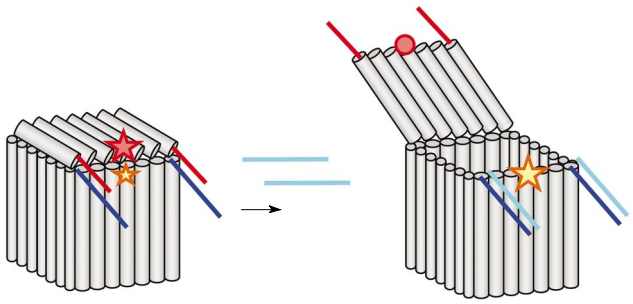


Figure 23. DNA box with opening lid equipped with the 'key–lock' system and the state detection system.[161]

$10^4 - 10^5$ nm^3. Thus, the obtained DNA aggregates have a rather large cavity for accommodating various biomolecules. The nucleotide shell of such capsules protects the material placed inside from destructive action, thus preserving the integrity of the molecules carried therein.

The design of a DNA box with a lid functionalized by a 'key–lock' system was reported (Fig. 23).[161] The opening lid is one of the faces of the cubic DNA box. The 'lock' is made of complementary oligonucleotide sequences attached to two adjacent faces. The complex of these oligonucleotides defines the closed box, functioning as the 'lock'. The single-stranded site protruding from the 'lock' duplex serves as a sort of keyhole. As the 'key' oligonucleotide complementary to both the 'keyhole' and one of the 'lock' strands is added to the solution, the 'lock' is unlocked upon displacement of chains in the 'lock' duplex. The box is thus opened. This DNA aggregate was also equipped with a state detection system. For this purpose, the lid and the front face of the box are modified with fluorophores that form a donor–acceptor pair (for example, dyes Cy3 and Cy5). Effective fluorescence resonance energy transfer (FRET) between the dye residues corresponds to the closed state of the DNA box where the residues are spatially proximate. In the open state of the DNA box, the distance between the fluorophores increases and no FRET takes place.

The fabrication of three-dimensional origami structures is an important step towards practical applications of the DNA objects. This Section demonstrated the potential possibility of preparing a DNA box with a lid that is opened in response to the presence of a particular substance (in this case, a specific nucleotide sequence) in the environment. The molecules carried inside the origami containers are almost completely isolated from the recognition and protection biological systems.

IV. Investigations of the structures of DNA nanoobjects

Primary characterization of monomeric DNA blocks or more complex associates is often performed using indirect methods for structure determination. Rapid tests are made possible, for example, by placing particular DNA fragments acting as restriction endonuclease recognition sites in the blocks or in the final assembled objects. The DNA structures labelled in this way are cleaved to give a predictable set of products, which indicates the quality of the assembly.[114, 118, 123, 163] The step-by-step assembly of the DNA structure can be readily monitored by gel retardation assay,

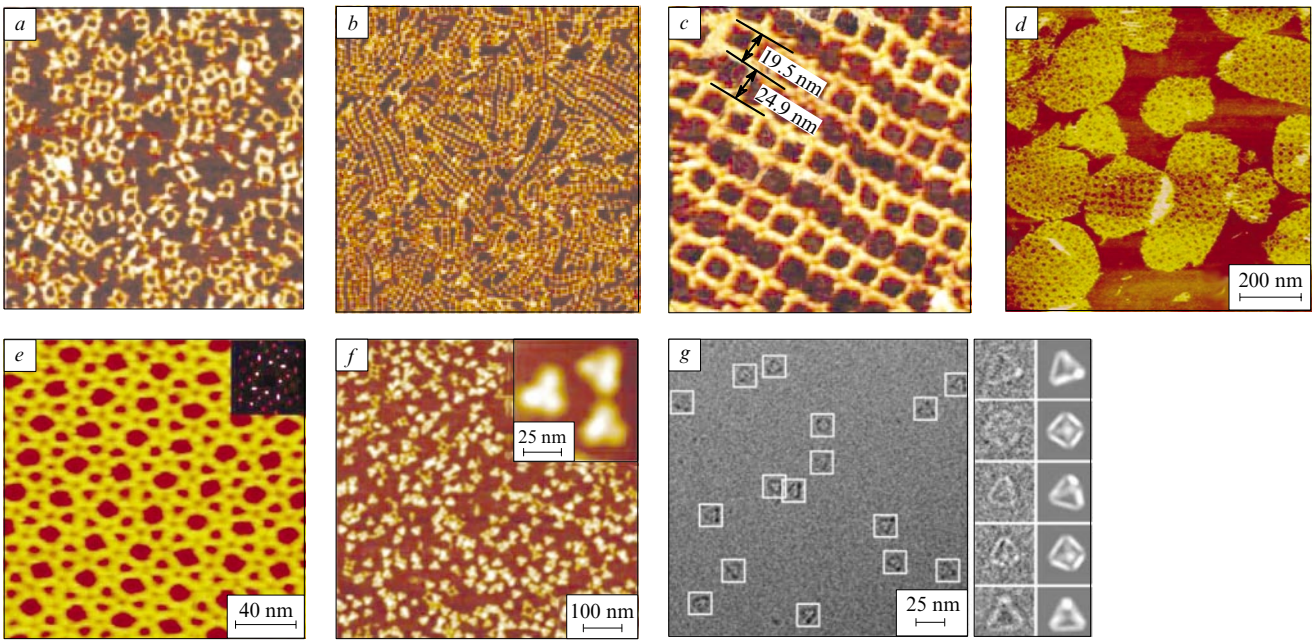


Figure 24. Typical atomic force (*a*–*f*) and cryoelectron microscopy (*g*) images of DNA objects: DNA polygons based on the 4DA blocks (*a*–*c*),[109] periodic patterns based on the 5DA blocks (*d*, *e*),[34] DNA tetrahedra based on the 3DA blocks (*f*, *g*).[33]
Scanning area: 500 × 500 nm (*a*), 1000 × 1000 nm (*b*), 200 × 200 nm (*c*).

which detects changes in the size and/or shape of the association product.[34, 101, 114, 119, 125]

In addition, DNA objects are often investigated using methods requiring pre-modification of DNA structures. For example, the introduction of fluorophore molecules makes it possible to obtain fluorescence microscopy images of the object. This is used most often to characterize periodic DNA patterns.[32, 131, 133] Labelling of particular oligonucleotide components by two fluorescent dyes is used to determine the degree of their proximity in the resulting DNA object by investigating the efficiency of FRET. A procedure of this type was described to prove indirectly the structure of a planar DNA polygon [73] and periodic DNA patterns.[164]

Accurate determination of the structure and shape of a DNA object is possible only using more complex instrumentation. A most common method used to prove the structures of nanoobjects is atomic force microscopy (AFM). The images and dimension characteristics of the vast majority of described nano- and micro-sized DNA structures were obtained by this method (Fig. 24).[31, 33, 91, 109, 128, 149, 151] However, AFM is not always applicable to the investigation of three-dimensional structures based on biological molecules. Cryoelectron microscopy was found to be more appropriate for investigating the spatial organization of three-dimensional DNA objects, for example, polyhedra (see Fig. 24).[33, 161, 165] In addition, an electron microscopy technique for investigating the structure of three-dimensional DNA nanotubes was reported. However, this requires introduction of metal nanoparticles into the DNA object to achieve the image contrast.[31, 138, 144] Using electron microscopy, the three-dimensional origami structure was proved.[162]

V. Prospects for the practical application of DNA nanostructures

Despite the great diversity of the obtained DNA nanostructures, most of them still serve for demonstration purposes, in particular, those reflecting the aesthetic sense of the authors. Only prospects of future practical applications of the DNA architectures are mainly discussed in the literature. According to many authors, the greatest potential for their application is related to the possibility to obtain hybrid architectures bearing non-nucleotide components along with DNA-based elements. An important feature of this decoration of DNA structures is the possibility to place functional groups and elements into strictly specified positions relative to one another and of the surface as a whole. A multitude of diverse modification methods for DNA objects with metal cations,[31, 166–174] nanoparticles,[110, 175–181] quantum dots,[182] proteins [31, 132, 183–188] and so on have been proposed.

Several prospective application areas for modified DNA structures can be distinguished. One is related to decoration of DNA molecules with metal ions or nanoparticles, *i.e.*, to the design of DNA nanowires.[31, 166, 168–174] The pioneering study [170] reported a two-stage metallization of the DNA chain: first, the DNA molecule was attached by its ends to two gold electrodes; the Na^+ counter-ions that counterbalanced the DNA charge were replaced by Ag^+, and then the NA chain was completely coated by silver metal in an acidic solution of hydroquinone under dim light. In later works, the metallized DNA fragments were obtained by treating modified DNA bearing aldehyde groups with a solution of $AgNO_3$ or Tollens' reagent (ammoniacal silver nitrate) followed by treating with AuSCN in hydroquinone. These procedures result in the formation of NA wire completely coated with gold.[31, 166, 171, 172] A similar procedure was utilized to metallize a nucleic acid–protein filament (DNA-RecA) [166] and periodic DNA lattice.[31] The length of DNA nanowires is several micrometres and the diameter is $\sim 50-100$ nm.

Another method proposed for metallization of NA analogues includes the fabrication of a right handed helix by two chains of NA-like molecules around the central axis formed from metal ions. The procedure is based on the ability of metal cations to form coordination compounds (complexes). For example, Cu^{2+} and Hg^{2+} ions can give stable complexes with two 3-hydroxy-2-methylpyridin-4(1*H*)-one and pyridine molecules, respectively. When placed into a solution of Cu^{2+} and/or Hg^{2+}, nucleic acid analogues containing one or both of these ligands instead of heterocyclic bases form a modified DNA duplex around the axis saturated by metal ions. This duplex is stabilized through coordination interactions instead of hydrogen bonds.[168, 169]

A potential use of DNA nanostructures is related to the possibility of placing various biomolecules, NA fragments or proteins, on their surface. The methods of attachment of these components are based on the use of complementary recognition, specific antigen–antibody or biotin–streptavidin interactions, NA aptamers and on the formation of covalent cross-links through bifunctional linkers. Two-dimensional DNA tiles with NA fragments arranged on them in a strict order can be used as nano-sized analogues of DNA chips. A 90×60 nm DNA tile containing oligonucleotide fragments corresponding to three genes, *Rag*-1, *c-myc* and *β-actin*, in definite positions of the surface was obtained. The addition of RNA targets complementary to these gene regions to the solution gives rise to hybridization complexes, which can be detected on the surface of the DNA tile by atomic force microscopy.[189] The authors noted some limitations in the use of these DNA chips. The most significant one is high density of arrangement of specific areas on the nanochip surface with large numbers of NA probes. For example, it was noted that steric hindrance and electrostatic repulsion of the NA fragments on the tile surface can reduce the efficiency of capture of the target by NA probes located closer to the centre of the sensor structure.

Periodic DNA lattices were employed to fabricate the DNA chip for simultaneous detection of nucleic acids, proteins and other organic molecules in multicomponent systems.[190] The DNA chip was designed from three blocks: the A1 and A2 blocks were modified by organic dyes Cy5 and Red-X, respectively, and the B block had a single-stranded sensor site that formed a signal complex with the complementary oligonucleotide labelled by the fluorescent dye Fluor 488. This site in the free state is a probe that either interacts specifically with the protein or organic molecule or it is complementary to a particular site of the gene. Upon the addition of the corresponding target molecules to the DNA chip, a complex is formed, while the signal oligonucleotide containing the fluorophore is displaced. The presence of these molecules is detected by the decrease in the fluorescence intensity of specific chip areas. Simultaneous identification of several targets was accomplished using two types of dyes attached to the A1 and A2 blocks.

When the DNA chip is assembled, the constituent blocks are mixed in the ratio (A1 + A2) : B = 1 : 1. However, the DNA chip for identification of several types of molecules is constructed from several monomeric DNA lattices, each containing the A1 and A2 blocks taken in a strictly definite ratio and specific B type blocks bearing a specified probe. Hence, the DNA lattices meant for identification of different molecules have characteristic different colours, which change in a definite manner upon the formation of the probe – target complex. The number of targets that can be identified in one system is determined by the sensitivity of the fluorescence detector. This concept was tested in relation to detection of four targets that sharply differed in their type: the DNA of two viruses (human immunodeficiency virus and the severe acute respiratory syndrome virus), the human α-thrombin protein and adenosine triphosphate.

The positioning of different protein molecules on the surface of one DNA structure opens up prospects for the preparation of so-called nanofactories. The proximate positions on the DNA tile of several enzymes involved in the series of successive transformations can accelerate multienzyme reactions in which the diffusion of substrates is the rate-limiting step. The early advances of the development of an artificial multienzymatic complex were reported.[191] The oxidoreductase and luciferase enzymes, which catalyze two coupled reactions, were attached to the DNA molecule using highly specific biotin – streptavidin interaction. The efficiency of substrate transformation in this complex was at least twice as high as that in the case of the simultaneous presence of these enzymes in solution. Indeed, even mere spatial proximity of two and more organic molecules with complementary oligonucleotides attached to them may accelerate the reaction between the reactants by switching the reaction to the pseudo-intramolecular type.[77]

Particular expectations for production of nanofactories are connected with implementation of the DNA origami type structures as the platform for their construction. These multimolecular associates provide the possibility of modifying a large number of components forming them; functionalization of a particular component specifies the position of this function on the surface of the item being formed and the mutual positions of the functions.

Three-dimensional DNA structures (DNA polyhedra and origami boxes) are considered as candidates for encapsulation and transporting of diverse molecules. For example, methods of packing of protein molecules [192] or nanoparticles [119] within such structures were described. An important feature of these DNA objects is that, on the one hand, they possess structural stability and, on the other hand, they can be opened upon biodegradation of the walls by nucleases [112, 118] or in the presence of specific signalling molecules in the solution.[161, 193, 194]

A DNA tetrahedron that changes its shape in response to the presence of a particular nucleotide sequence in the solution was designed.[194] For this purpose, a loop that opens and forms a complementary complex upon the addition of the corresponding oligonucleotide is inserted in one tetrahedron edge. As a result, the increase in one edge of the DNA polyhedron leads to an increase in the area of one its face, and this may result in the release of molecules that are transported inside this DNA container. The origami design of a DNA container equipped with a 'lock' opened in response to the presence of the oligonucleotide 'key' has been already described above.[161] Thus, three-dimensional DNA structures can be used to protect and transport specific molecules, including those targeted into cells.

The possibility of strictly definite arrangement of molecules on the surface of a DNA object can be used for studying its properties. For example, by arranging metal nanoparticles on the DNA lattice where the distance between the modified sites is strictly specified, it is possible to study their optical, electronic and catalytic characteristics. The unique shape of DNA nanotubes allows them to be used for a sort of packing and subsequent NMR study of the structure of transmembrane proteins.[155]

In relation to a DNA molecule and a two-dimensional DNA lattice, it was shown that DNA-based structures are suitable as matrices for molecular lithography.[195] For this purpose, DNA objects supported on mica surface were coated by a 20 nm-thick gold film and then the metallic film was glued to a glass plate. After separation of this sandwich structure, the outer surface of the gold film that remained on the glass showed the negative image of the DNA lattice that was initially formed on mica.

Yet another potentially important field of application of DNA nanostructures, in particular, two- and three-dimensional lattices, is for the preparation of molecular sieves or molecular filtres. Three-dimensional DNA lattices with $\sim$ 9-nm inner pores were found to be able to adsorb protein molecules.[141] In these experiments, DNA lattices were treated with a set of proteins with different molecular mass that are negatively charged at neutral pH. After long-term incubation, the DNA lattices were washed out and the proteins that had been linked to the DNA structure were analyzed. It was shown that only proteins with molecular mass of < 45 kDa are linked to the DNA lattice.

Thus, quite a few investigations were aimed at a variety of practical application of DNA nanostructures in electronics, medicine, nanomechanical systems and so on. However, there are no works on actual application of the DNA-based structures; they still remain only prospective elements of molecular tools and future technology.

VI. Conclusion

The structural DNA nanotechnology can undoubtedly be regarded as a new chapter in the history of DNA molecule. The bottom-up approach to the design of nanoobjects applied in the DNA nanotechnology allows for fabrication of objects with intricate shape or a sort of architectures based on linear DNA molecule. Using only Watson – Crick base pairing for the assembly of monomeric blocks, it is possible to construct static and dynamic, discrete and periodic, one-, two- and three-dimensional DNA structures. The success in the fabrication of various objects attests once again in favour of DNA as an ideal building block for nanometre-sized objects. For getting unique properties and for extending the scope of structural DNA nanotechnology, additional structural elements, for example, proteins or organic residues, are often introduced in NA blocks.

The large diversity of shapes of the fabricated objects and the possibility of decorating the constituent oligonucleotides without changing their self-assembly behaviour open up fascinating prospects for the future practical use of DNA nanostructures. In the development of DNA nanotechnology, most promising is the preparation of transport containers and scaffolds for biomolecules, design of the

medical diagnostic platforms and components of micro- and nanoelectronics.

This review was written with the support from the Russian Foundation for Basic Research (Project No. 10-04-01492-a), Integration Grants of the Siberian Branch of the RAS (39, 76), Presidium of the RAS Programmes 'Molecular and Cell Biology' and 'Grounds of Fundamental Research of Nanotechnologies and Nanomaterials' and by the Ministry of Education and Science of the Russian Federation (GK No. P1073).

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