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Review

Building objects from nucleic acids for a nanometer world

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Abstract

Nucleic acids are an ideal material for the construction of nanometer-scaled objects. An overview is given which focuses on the structural aspects of this field of research using native DNA and RNA and especially also chemically modified derivatives, which offer structural elements other than the Watson–Crick interaction. First examples for applications are discussed.

Keywords: DNA nanoarchitectures; Bottom-up approach; Spatial arrangement; Self replication

1. Introduction

The past 50 years have been years of enormous technological and scientific changes. Most individuals in our part of the world have at least one computer and the rise of the internet has opened possibilities few people would have foreseen 50 years ago. The same is true for the advent of mobile communication.

On the other hand, we have learned to understand hereditary processes and know the essential mechanisms of how nature stores information in the form of DNA [1,2]. We have learned to synthesize DNA and also RNA [3], and have found out that nucleic acids are much more than nature's hard drive and memory but rather have a rich world of their own: not only are they readable and replicable but also catalytically active [4]. In addition, huge libraries of nucleic acids can be generated out of which aptamers with almost any property can be selected entirely in vitro [5–8]. Now, when trying to build ever smaller structures and devices it might turn out that the metaphors used before will in fact become reality and that both mentioned fields of progress will not be independent of each other anymore. The reason is that the major technology for the generation of miniature objects—for example in computer chips—is photolithography where light-responsive layers are irradiated in a structured fashion using photo masks. In the subsequent process the two-dimensional pattern of the mask is then transferred to the material underneath. Using multiphoton processes it is even possible to create complex three-dimensional structures with features as small as 100 nm [9]. However, this technology has a lower limit in terms of the smallest structure that can be generated and this is understandably related to the wavelength of the light which is used. Instead of continuing to try and make structures smaller one can also try to cross this apparent technological barrier by building the desired structures from still smaller subunits in a molecular bottom-up approach. Not all types of molecules are equally well suited but nucleic acids have many features that make them an ideal material from which defined, nanometer-scaled objects can be constructed [10]. First, the dimensions are perfectly suited for a nanometer world: the typical B-type double helix has a diameter of around 2 nm and approximately 10 base-pairs form one helical pitch with a height of 3.4 nm. The duplex strand has

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a persistence length of ca. 50 nm—a measure to characterize the stiffness regarding bending of the helix axis [11]. Second, established techniques are available to generate and manipulate long DNA strands and to equip them with additional chemical functionality [12–17]. Third, these polymers are made from monomeric nucleotide units which can be arranged in an arbitrary sequence. This sequence provides a reliable "addressing space" for the programming of interactions of single strands. Furthermore, interesting options arise from the potential replicability. An overview of all the structural or functional objects that have been constructed from nucleic acids up to now is beyond the scope of this review. We will focus on discussing some of the highlights in the field. For reviews with different scope and focal points we refer to the literature [10,18–21].

2. DNA nanoarchitectures based on DNA duplexes

One problem to overcome with DNA as material is that double-helical DNA is a linear polymer, which makes it difficult to build stable three-dimensional objects. As a consequence, branching points are needed. One such branching point is the "Holliday junction". This construct has been long known and was first used to explain gene conversion events in hereditary processes [22]. Topologically, this type of junction could already be used for DNA nanoarchitectures. However, due to the sequence symmetry the branching point is able to move in these junctions. In a seminal paper, Seeman showed how to obtain stable junctions that are suitable for the construction of DNA objects (Fig. 1) [23] which paved the way for many studies to come. Using this approach he constructed, for example, the first closed polyhedral object from DNA which had the connectivity of a cube (Fig. 2) [24]. In this assembly the aforementioned junctions—in this case three-way junctions—form the eight vertices of the cube.

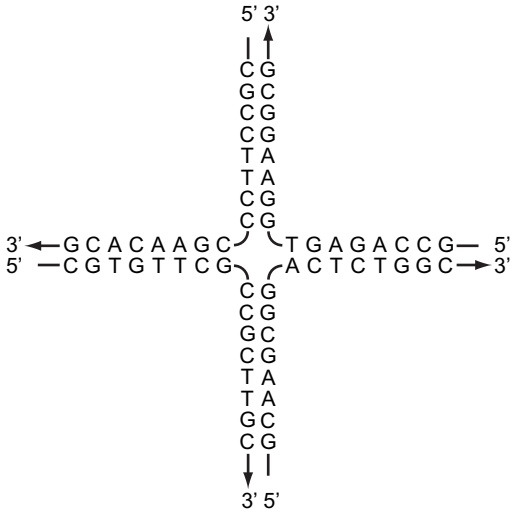

Fig. 1. Example of a 4-way DNA junction [23]. Unlike a "Holliday junction" [22] this type of junction—conceived by Seeman—is "immobile" due to its designed sequence and thus provides a stable branching point which can be used for building DNA architectures.

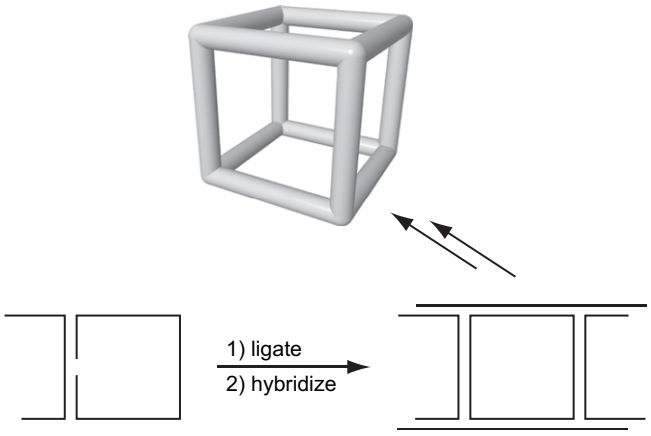

Fig. 2. Construction of a DNA object with the connectivity of a cube [24]. Each edge is in fact a double helix but the helicity aspect is omitted for clarity. Lines represent single oligonucleotides.

Each face of the cube is formed by a circular single strand which is catenated to its four analogues forming the neighboring faces (the aspect of catenation is omitted for clarity in Fig. 2). However, it is not effective to just combine the constituting oligonucleotides. The cube was rather assembled following a convergent reaction scheme: at first one oligonucleotide strand was cyclized using another oligonucleotide as a template. After ligation, to form the first covalently closed ring, more strands were added to form a square assembly with four sticky ends (as shown in Fig. 2). Two of these were assembled and ligated to form a three-square-building block, etc., until the cube was finally obtained. The overall yield of the synthesis was about 1%. The connectivity was proven by gel electrophoresis after digests using individual restriction enzyme cleavage sites which were introduced into the edge-forming oligonucleotides. Before cleavage, the DNA cube was a denaturation-resistant polycatenane.

Later, Seeman's group also constructed a much more complicated truncated octahedron (Fig. 3) [25]. This polyhedron had eight hexagonal and six square faces. Again, the vertices were formed by junctions and each edge was formed by two turns of double-helical DNA (the aspect of helicity is omitted in Fig. 3 for clarity). At first, the square faces were assembled by ligation of four branched oligonucleotides with four-way junctions. The remaining four "outer" arms of this square building block were, however, not sticky ends but hairpin structures. Each arm had a cleavage site for a different restriction endonuclease (exemplarily indicated in one case as a gray box in Fig. 3). This strategy is similar to the protecting group strategy in synthetic organic chemistry because when desired, individual arms could be digested to sticky ends which could then be used for further ligation steps. Another feature in this study was that the assembly was performed on solid support using all the advantages of this technology. For example, products resulting from failed ligations could be separated by denaturation, etc. The immobilization was performed via an unused arm of one of the junctions in the first building block. This part was ligated to a solid support with a complementary end. The overall complex was made from an impressive

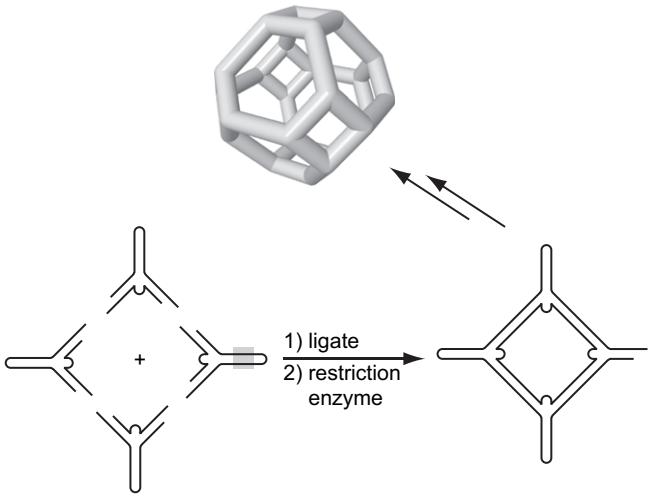

Fig. 3. Construction of a DNA object with the connectivity of a truncated octahedron [25]. Each edge is in fact a double helix but the helicity aspect is omitted for clarity. Lines represent single oligonucleotides. Vertices are four-way junctions—one of the four arms remains unused. Hairpin structures with individually addressable restriction enzyme cleavage sites (gray box) serve as ''protected'' sticky ends. The assembly was performed in a solid-phase approach.

number of 2550 nucleotides, weighing 790 kDa. Finally, the topology was again proven with different restriction enzyme digests. It is important to note that the overall shape of the assembly was not analyzed but rather its connectivity, as was the case in the previous example. It is even unlikely that both objects exist exactly in the conformation shown in Figs. 2–3 because of the flexibility of the assembly.

To obtain shape-persistent objects Turberfield et al. constructed DNA tetrahedra [26]. Fig. 4 shows how the tetrahedra can be obtained from the ligation of four oligonucleotides. For the synthesis all constituting parts were mixed in equimolar quantities at 95 °C. Then the solution was cooled to 4 °C within 30 s, affording the tetrahedron in an impressive 95% yield. Ligation of the product to form a closed polycatenane was possible. The correct connectivity was again proven in digestion experiments. However, in this case the structure was backed-up by atomic force microscopy (AFM) measurements. This technology also allows the measurement of forces via the AFM tip. Compression experiments with the DNA tetrahedra confirmed the expected rigidity. Via the introduction of

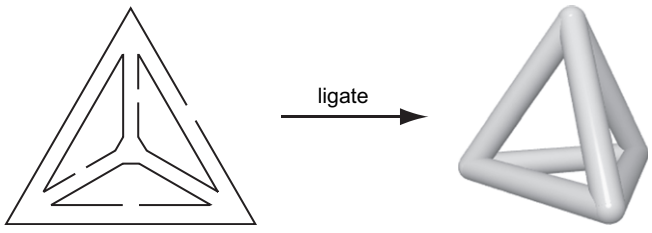

Fig. 4. Construction of a DNA tetrahedron [26]. Each edge is in fact a double helix but the helicity aspect is omitted for clarity. Lines represent single oligonucleotides.

single-stranded gaps it was possible to ligate two tetrahedra with a connecting oligonucleotide that bound to the respective gaps. The rigidity of the setup allows discrimination of the inner and outer part of an edge-forming duplex. Thus, the position of the gap determined whether dimer formation was allowed or not. However, the assembly of the tetrahedron is possible in two diastereomers (not enantiomers because of the unchanged chirality of the constituting oligonucleotides). What is ''inside'' in one diastereomer is ''outside'' in the other. With dimerization experiments, Turberfield et al. could clearly show that the formation of the tetrahedral assembly occurs with high diastereoselectivity. In a following study Turberfield et al. used this tetrahedron as a cage for a guest molecule [27]. They calculated that the central cavity should be able to accommodate a globular protein of about 60 kDa with a radius of ca. 2.6 nm and chose cytochrome *c* for their studies. The protein was labeled with an oligonucleotide which could hybridize to a single-stranded gap as described before. Again the position of the gap determined whether the protein ended up inside or outside the tetrahedron. Recently the Turberfield group has also presented a self-assembled trigonal bipyramid made from DNA [28].

A particularly intriguing topological structure is the family of the so-called ''Borromean rings''. The simplest representation of this topology is shown in Fig. 5A: the three rings are interlocked and cannot be separated. However, cleavage of any one of these rings liberates all three of them. Mathematically, Borromean rings have an equal number of positive and negative crossings at specific positions. Using B-DNA and Z-DNA with opposite helical sense Seeman et al. were able to generate a setup with three cyclic oligonucleotides which had the desired topology [29]. Fig. 5B gives a representation of this setup. In contrast to the previous figures, the single strands are drawn as tubes in this example.

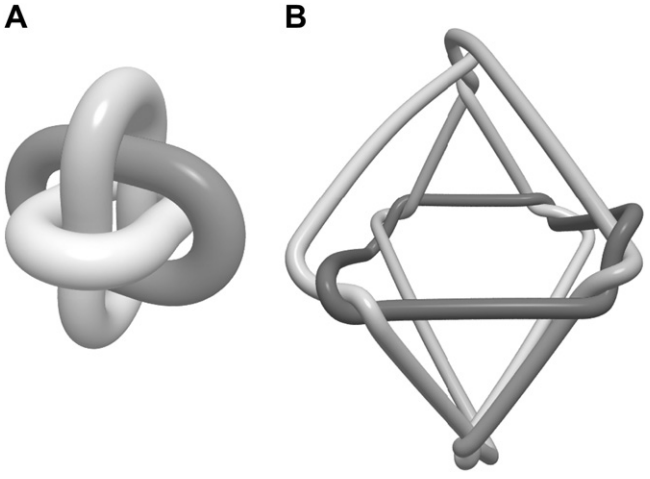

Fig. 5. (A) The simplest version of ''Borromean rings''. This drawing serves only for demonstration purposes but is not an architecture that has been realized already in the drawn state. (B) Seeman's version of a DNA object having the topology of Borromean rings [29]. In this representation each tube represents a single oligonucleotide chain. These representations have been generated using KnotPlot [111].

In principle, DNA as material offers the potential to replicate the structures using polymerases but for topological reasons this is not possible for structures like the already mentioned cube or the truncated octahedron. Joyce et al., however, succeeded in constructing a DNA object which can be replicated [30]. Therefore, they designed a 1669 nucleotide-long sequence which—in the presence of five 40-mer helper oligonucleotides—folds to form an octahedron (Fig. 6, top). This object forms by the assembly of subunits called PX motives [31]. In the PX motif two separate DNA helices intertwine sequence-selectively to form a rigid unit (Fig. 6, bottom). Thus, for example, both units labeled "PX1" in Fig. 6 are actually double helices which can interact with each other but not with any other PX motif ("paranemic cohesion" [31]). The way in which this is achieved is shown in the bottom part of Fig. 6. As can be seen in this particular PX motif, six crossovers of DNA strands occur. The remaining blocks are labeled "DX" and correspond to so-called double crossover motives [32] (Fig. 6, bottom). These contain the short helper oligonucleotides (drawn as thick gray lines). Thus most of the octahedron is still assembled from one single long oligonucleotide. The structure of the entire complex was investigated with cryo-electron microscopy and the assembly of hollow octahedra of 22 nm diameter could clearly be proven.

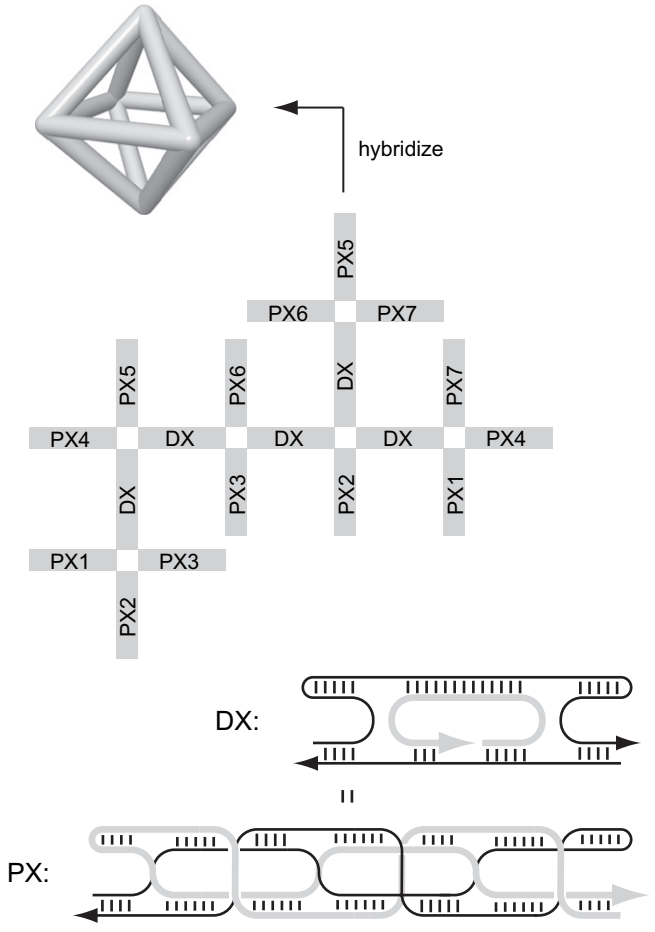

Fig. 6. Assembly of a replicatable DNA octahedron from a 1669 residue single-stranded nucleic acid and five helper strands [30]. Each of the gray blocks corresponds to a DX tile or to one-half of a PX DNA tile. The structures of both tiles are shown in the bottom. Each line corresponds to a single strand and the arrow points in the direction of the 3′-end. The helper strands are incorporated in the DX tiles (gray line). The two corresponding halves of a PX tiles (both labeled for example PX1) combine sequence-selectively to form the edges. Again the helicity aspect in the double-stranded regions is omitted for clarity.

In a seminal contribution, Rothemund showed that the strategy of using one long DNA single strand and several short strands can be used to generate virtually any desired shape [33] (Fig. 7). He called his approach "DNA origami": the (two-dimensional) shape to be generated is filled line-by-line with the long single strand going back and forth. The short strands, which were called "staple strands", hold the long strand in place. The generated structures were characterized by AFM and include stars, smileys and even banners with text on them or representations of a world map. Additional contrast on the tiles in the AFM images was obtained by the use of dumbbell hairpins protruding from the planar structure.

Instead of one single strand going through the entire complex, one can also assemble larger structures by pasting together several of the previously mentioned interwoven DNA duplex patches or "tiles". Apart from the already shown double crossover "DX" tiles of which there are many more than the presented one [34] and the paranemic cohesion "PX" tiles, there are, for example, also triple crossover "TX" complexes or tiles [34], etc. Using these tiles Yan et al. have constructed what they called a "DNA nanoarray" [35] (Fig. 8A). Instead of a random association, it is built from 13 individual tiles (labeled A–M in Fig. 8A) which each take up a well-defined position in the array. This 5×5 array has C_2 symmetry and each tile has the same core sequence but different sticky ends. At first the individual tiles were pre-hybridized and then they were combined in the correct stoichiometric ratio. For different approaches to generate

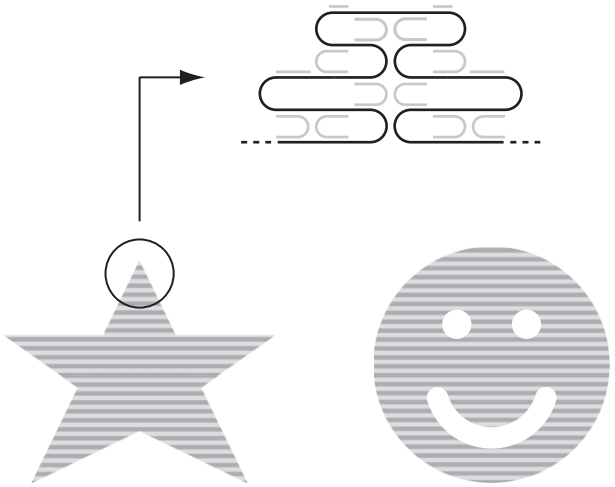

Fig. 7. Generation of arbitrary shapes by DNA origami [33]. The pattern is created from a long oligonucleotide strand (black line) which goes through the figure line-by-line and is held in place by "staple strands" (gray lines). Thus stars, smileys and many more shapes were generated.

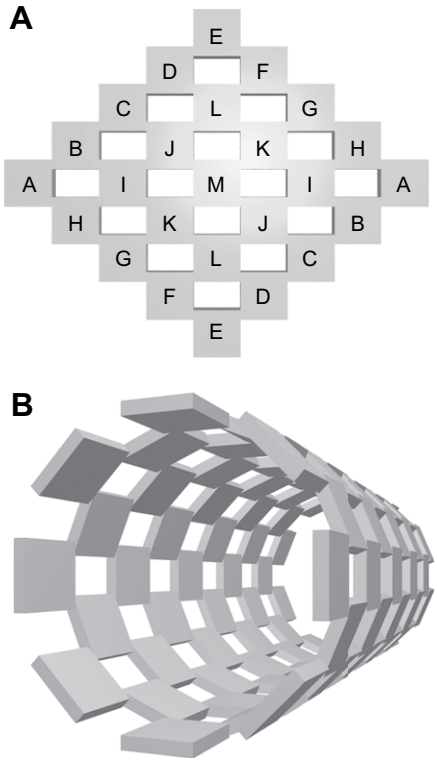

Fig. 8. (A) A 5 × 5 array of 13 individual DNA tiles [35]. (B) A tube constructed from DNA tiles [36].

DNA arrays, see especially Section 3 about DNA architectures for spatial arrangements.

LaBean et al. used triple crossover tiles to construct tubes of ca. 25 nm diameter and up to 20 μm in length (Fig. 8B) [36]. The ''ring closure'' of the tube was favored by the introduction of thiol groups: one half of the tiles had thiol-modified hairpin structures protruding perpendicularly to either side of the tile. Conditions were chosen which favor the formation of disulfide bridges, introducing a curvature in the array. In a control experiment the presence of DTT to prevent disulfide formation led to sheet-like structures instead. A so far unmentioned feature of DNA is that it can also be metalized (see for example [37]). Thus, DNA nanowires could be generated. The dimensions of these nanowires make them ideal for bridging the interface between the world which is accessible via photolithographic methods and the ''nanoworld'' below. Conductivity measurements yielded bulk resistivities of 14–30 μΩ/m which is still relatively high. Progress towards metallized DNA was also achieved by the group of Shionoya [38–40] and by the Carell group, who used DNA templates with synthetic base-pairs capable of complexing transition metal ions [41–44]. This approach allowed the controlled stacking of 10 transition metal ions inside a DNA duplex [43].

Rothemund et al. also created DNA tubes [45]. They used double-crossover tiles in an array with an inherent curvature. Fygenson et al. addressed the fragility of these types of tubular constructs which sometimes melt at temperatures below 40 °C and can disintegrate in deionized water due to the presence of nicks [46]. These nicks result from the many single oligonucleotides contributing to the overall structure. Therefore, Fygenson et al. tried to apply T4 DNA ligase and found out that nicks between the tiles could be healed—affording fortified DNA tubes of increased mechanical stability.

3. DNA architectures based on additional structural elements and RNA architectures

The previous section has already shown many examples of what can be obtained if, more or less, only the regular Watson–Crick interaction of DNA duplexes is used. But the repertoire does by no means stop here. Another interaction motif of nucleobases—different from the one in the duplex—is, for example, the G-quadruplex interaction, as excellently reviewed previously [47]. In principle this interaction can be used in very much the same way as the already mentioned crossovers, except for the fact that the G-quadruplex interaction is more dependent on buffer conditions, which can be used for the conditional formation of crossovers. It could even be shown that it is possible to let non-identical G–G domains interact ''self-specifically'' to form only two homodimers as opposed to heterodimers [48]. In addition, G-quadruplex-based RNA structures were used to control ribozyme activities and for the modulation of gene expression [49,50]. Moreover, Willner and colleagues have recently reported an elegant approach of using rolling circle amplification (RCA) to create linear DNA chains containing G-quadruplex DNA aptamers that bind thrombin or both thrombin and lysozyme. These protein–DNA nanocomposites can organize themselves into supramolecular architectures that can serve as templates for immobilizing gold nanopartices via the amino groups on the assembled proteins [51].

Yet another idea is to use RNA instead of DNA to build up architectures. RNA is more labile but offers a wider range of tertiary motifs and possibilities for specific interactions [52]. The major contributions to the field of RNA architectures originated in the Jaeger group which coined the word ''RNA tectonics'' [53]. They used for example the so-called ''11-nucleotide motif'' receptor which binds to GAAA tetraloops [54,55] (Fig. 9A). Thus, it could be shown that ''RNA tectons'' contain hairpin tetraloops and tetraloop receptor sites that can form one-dimensional self-assemblies [53]. It is important to note that this interaction requires magnesium ions. This adds an additional level of control for this interaction. In follow-up studies the Jaeger group elaborated this approach [56,57] and added the so-called kissing-loop interaction to their repertoire [58]. Harada's group used a similar approach in what they called ''RNA LEGO'' [59]. In the kissing-loop interaction unpaired nucleobases of RNA hairpin loops form base pairs. Jaeger's work culminated in a programmable ''RNA jigsaw puzzle'' [60] (Fig. 9B). Here, two RNA hairpins were connected via a ''right-angle motif'' providing a 90° angle between the two stems. The loops of the hairpins provided four specific kissing loop interactions (labeled A/A′, B/B′, etc. in Fig. 9B). Four of these angular units form a tectosquare. 3′-Sticky ends were attached to program the interaction of the tectosquares. The direction into which these ends are

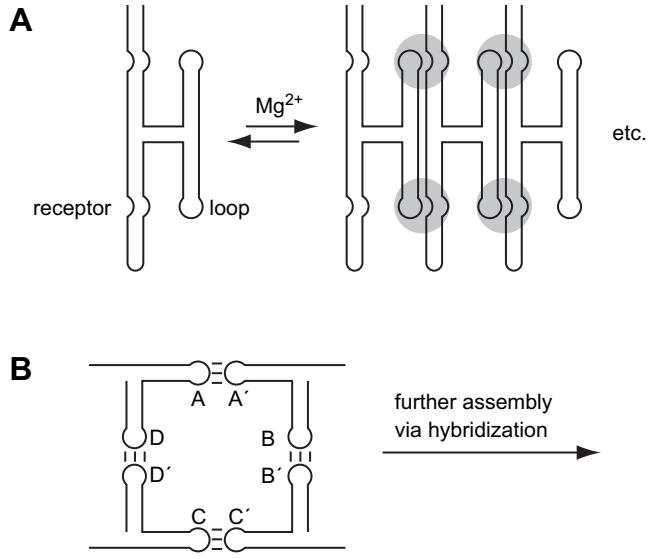

Fig. 9. RNA tectonics according to Jaeger et al. [53]. Top: the lines represent single-stranded RNA with GAAA loops and receptor regions which interact upon addition of Mg^{2+}. The interaction regions are highlighted with gray circles. Bottom: RNA tectosquares form from four angular subunits via kissing loop interactions (three dashes). These squares can be arranged via sticky ends to form higher-order aggregates or "jigsaw puzzles" [60].

presented can be chosen from two possibilities by swapping the right-angle motif. The sticky ends were used to generate higher-order aggregates like different lattices or ladders.

Going further away from the recognition properties of the nucleobases there are more ways to interact with a DNA duplex. One such way is the use of intercalators. We have recently shown that it is possible to modify DNA circles as versatile and rigid building blocks for architectures with custom sequences which can bear DNA modifications like, for example, anthracene [61] (Fig. 10A). Therefore we constructed circular double-stranded DNA with 168 nucleotides by ligation of fragments. These fragments were already curved due to an ordered arrangement of A-tracts. Via a 21 nucleotide wide single-stranded gap region it was possible to attach a functionalized oligonucleotide which had anthracene moieties attached to the 5-position of thymidine residues. In another recent contribution from our labs we have also used the kissing loop interaction to sequence-selectively combine DNA circles [62] (Fig. 10B). Again we used DNA circles with gap regions but this time RNA sequences were applied to close the gap. These RNA sequences were designed to form hairpin structures with complementary kissing loops. The formation of complexes mediated by the kissing interaction was proven in electrophoretic mobility shift assays. As expected, the complex was only formed in the presence of magnesium ions.

An entirely different approach uses the power of artificial DNA-binding polyamides [63]. These polyamides consist of residues derived from *N*-methyl pyrrole and *N*-methyl imidazol and can bind to the minor groove of DNA (Fig. 11). Importantly, they recognize the groove sequence-selectively, and with high affinity, according to well-established selection rules

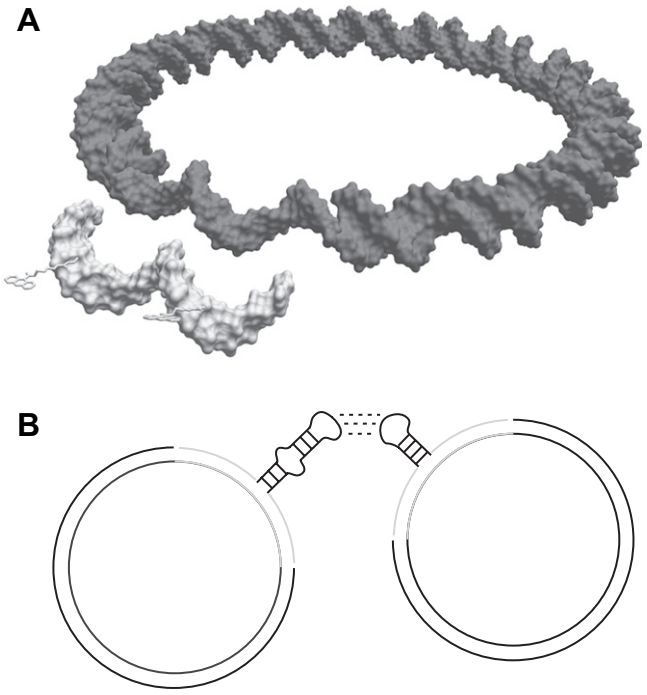

Fig. 10. (A) DNA circles with single-stranded regions can be customized with oligonucleotides bearing functional groups for orthogonal interactions with DNA duplexes—like for examples intercalators [61]. (B) Alternatively, the single-stranded regions can be filled in with RNA sequences forming hairpin structures which are capable of forming kissing-loop interactions [62].

[64]. We have linked two of these DNA-binding polyamides with a flexible linker and created what we called a "DNA strut". The strut was capable of gluing two DNA helices together in a sequence-selective fashion (Fig. 11). In our first

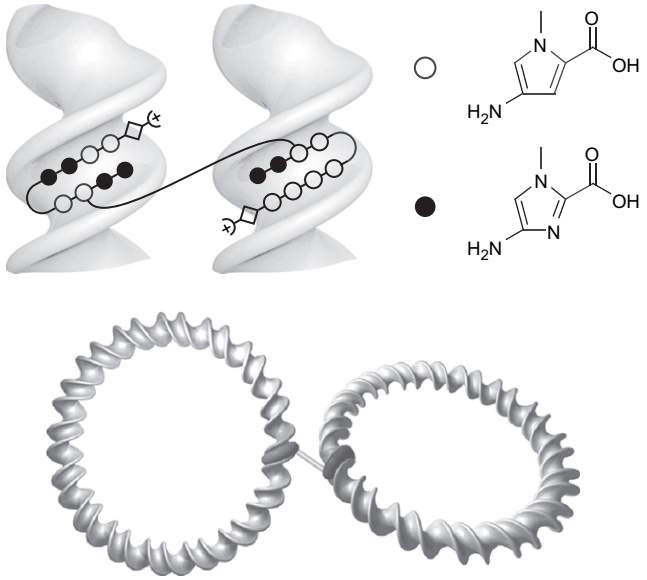

Fig. 11. DNA-binding polyamides as second structural element for DNA architectures (cf. Fig. 14). The polyamides are drawn in a ball-and-stick cartoon fashion. The circles correspond to the residues shown in the top right corner. Two of these polyamides form a strut which can sequence-specifically glue together for example two DNA circles [63].

example, two DNA circles were held together by one DNA strut which contributed only about 1% of the mass to the overall 208 kDa complex. Slight variations in the binding site led to a complete loss of the otherwise rather strong interaction ($K_D = 30$ nM).

As already mentioned, efforts have to be made to use the linear nucleic acids as material for complex three-dimensional objects with edges and vertices. Von Kiedrowski et al. came up with an entirely different solution compared to the ones based on junctions discussed so far, namely by connecting three oligonucleotides via a structure normally used for constructing dendrimers. He created what is called "trisoligonucleotidyls" [65] (Fig. 12A). The dendrimer-derived core was coupled via a phosphoramidite in a standard oligonucleotide solid-phase synthesis. After deprotection, the solid-phase synthesis was continued, affording three identical branches after the branching point. In this way, branched oligonucleotides were obtained which can interact to form the structures shown in the lower panel of Fig. 12A. Due to the resemblance of the branching point to a tetrahedral sp^3 carbon center, these aggregates were called nano-acetylene or nano-cyclobutadiene. In a follow-up study, von Kiedrowski et al. showed that the trisoligonucleotidyl approach is suitable for replication (Fig. 12B) [66] by using a different core derived from a trialdehyde. When this unit was combined with three 5′-hydrazide-modified oligonucleotides of different lengths a mixture of all possible products was formed. However, when a templating trisoligonucleotidyl compound with three different sequences complementary to the oligonucleotides A, B and C was added, only one single trihydrazone product formed, containing one A, B and C chain each. This represents already one half of a replication cycle. Similarly, Endo and Majima have pursued

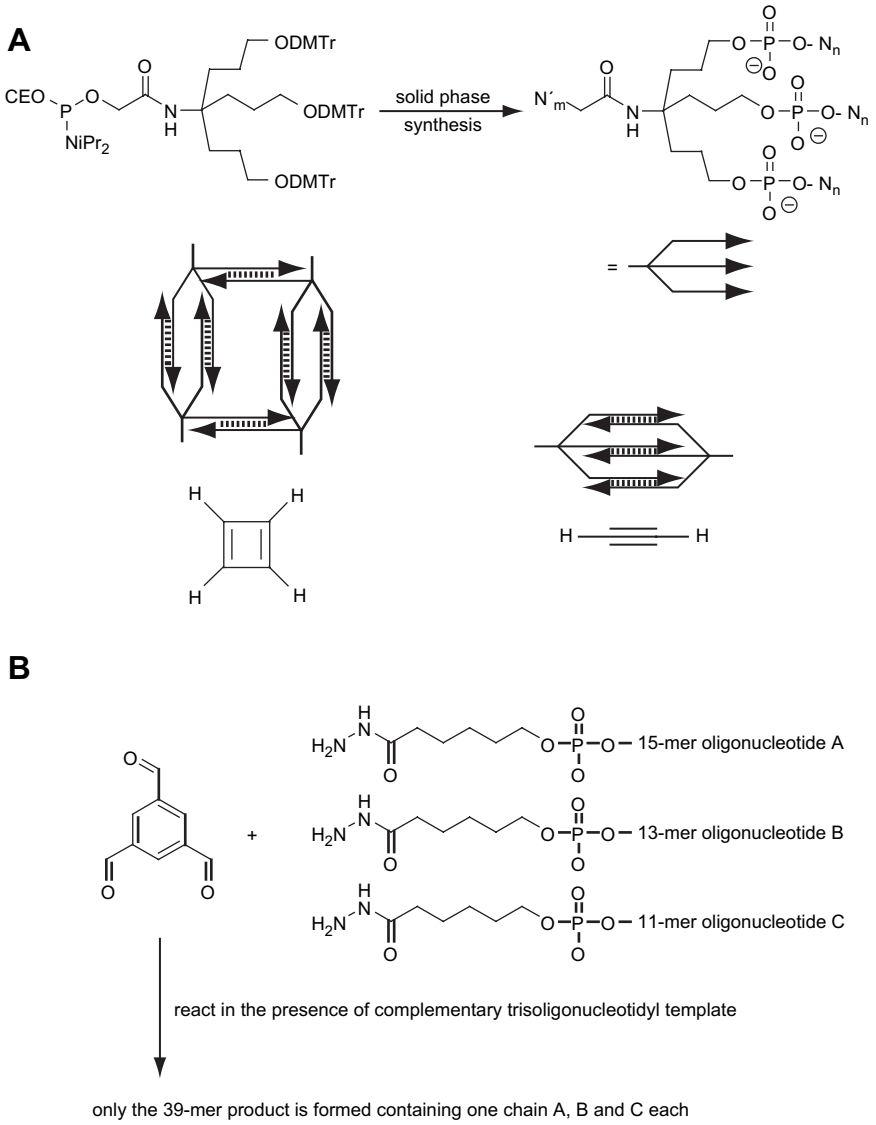

Fig. 12. (A) Branched "trisoligonucleotidyls" can be obtained by introduction of the shown phosphoramidite in a regular DNA solid-phase synthesis. After this residue three identical branches grow instead of one. The resulting units can be used to assemble objects resembling the topology of for example cyclobutadiene or acetylene [65]. (B) In the presence of a complementary template the trialdehyde core unit forms hydrazones only with the matching oligonucleotides. This represents one-half of a replication cycle [66].

a related strategy but used a porphyrine core for constructing DNA tubes [67]. Yet another idea to obtaining junctions has recently been presented by Mao et al. Here, streptavidin which is known to bind four biotin residues was used as a junction to assemble four biotinylated DNA duplexes [68].

4. Application of DNA architectures for spatial arrangement

While the objects, which can be generated from DNA, are certainly esthetically pleasing and their assembly often an intellectual masterpiece, one could ask the question about their usefulness. A possible answer to this question is that DNA objects can be scaffolds to arrange other objects in space—objects such as biological macromolecules which will then be amenable for X-ray crystallography [10]. Alternatively, DNA objects could provide the lattice for the arrangement of nanoelectronic components [10]. Even nano-scaled ''factories'' can be envisioned which allow sequential reactions on substrates in a flow [69]. The work discussed so far clearly shows how powerful nucleic lattices can be for spatial arrangements because of the many different shapes which can already be built up. In every case an ''interface'' or ''connecting technology'' between what shall be arranged in space and the nucleic acid scaffold is needed.

One such technology, which provides a very powerful interface, is the aptamer technology [5–8]. The strength of this approach lies in the fact that aptamers are DNA or RNA strands which fold into defined three-dimensional structures [70,71] that can bind to almost any target molecule from small molecules [72–77] to peptides and proteins [78–82], but are still made from the same ''material''. Hence, when attaching aptamers to nucleic acid-based nanoarchitectures a homogenous material capable of specifically interacting with other 'ligands' is achieved. Moreover, this concept can be expanded towards catalysis if ribozymes, allosteric ribozymes or aptazymes [83–87] are applied. For example, a well-characterized aptamer is a DNA aptamer that binds thrombin and its core structure is a G-quadruplex [88]. Yan et al. have incorporated this aptamer in triple-crossover (TX) tiles (Fig. 13) which assembled into linear nanoarrays in which the aptamers were arranged at a 17 nm distance [89]. After addition of thrombin this protein arranged itself periodically on the line of tiles (analyzed by AFM). In a following study, Yan et al. extended their system to a more complicated tile system which incorporated two different aptamers [90].

Instead of selecting new aptamers, LaBean and Kenan et al. have used a DNA hairpin with freely chosen sequence and used the phage display technology for a ''reverse screening process'' to select a single-chain antibody against the DNA hairpin as a handle [91]. The antibodies obtained in this process did not have any function other than binding but the authors suggested their use as adaptor moieties between the DNA scaffold and functionally active proteins which can be coupled to the antibodies using well-established protein engineering technologies. This avoids having to select new

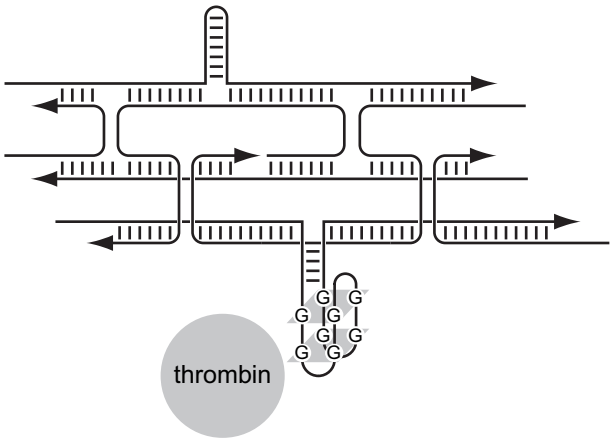

Fig. 13. Triple crossover (TX) tiles containing a thrombin-binding DNA aptamer with G-quadruplex structure. For reference reasons a hairpin structure was also present in the tile. These TX building blocks arrange in a linear nanoarray to which thrombin is recruited via the aptamer and thus arranged in a regular fashion [89].

aptamers for new target proteins and, instead, shifts the problem to protein–protein coupling.

Dervan et al. have recently shown that their system of DNA minor groove-binding polyamides (discussed in Section 3) can also be used to recruit proteins to tile structures (Fig. 14) [92]. Therefore, they derivatized the polyamides with biotin, which is able to bind streptavidin very strongly. As DNA architecture they chose layers of two different tiles. Only one of these tiles had a binding site for the polyamide. AFM analysis showed that half of the tiles were periodically decorated with streptavidin and, in particular, the distance between two streptavidins was again as expected.

The very strong and specific biotin–streptavidin interaction also stimulated other previous studies: LaBean and Yan et al. have modified grids and lattices very similar to the grid in Fig. 15 with biotin residues at the junctions and showed by AFM that streptavidin bound to the expected sites [69]. In a later study, Yan et al. presented what they called a ''molecular pegboard'' (Fig. 15) which consisted of nine cross-shaped tiles with individually addressable sticky ends (shown in black in Fig. 15) to which biotinylated oligonucleotides could bind. By choosing the required complementary sequence the place to which streptavidin was recruited could be chosen.

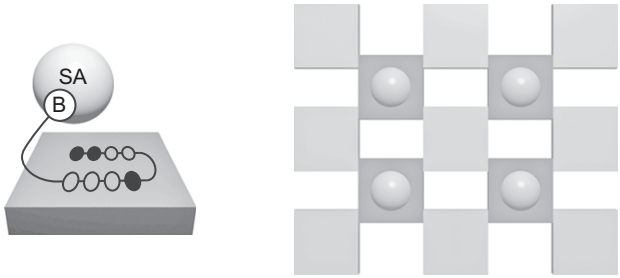

Fig. 14. Conjugates of DNA-binding polyamides with biotin can be used to recruit streptavidin sequence-selectively to only those tiles (drawn as gray boxes) of a DNA array carrying the polyamide recognition site [92]. For the drawing convention of the polyamides (cf. Fig. 11).

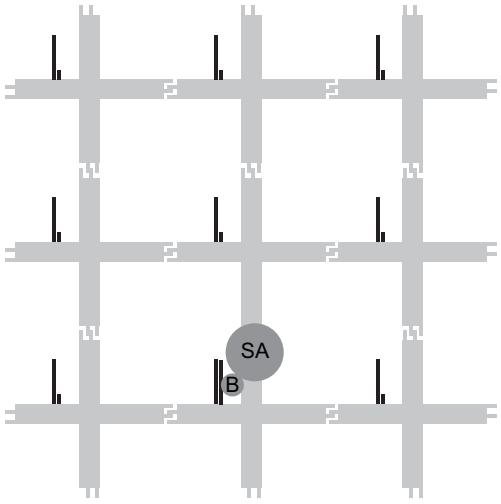

Fig. 15. A "molecular pegboard" according to Yan et al. [69] consisting of nine cross-shaped tiles (gray boxes) with individually addressable sticky ends (black lines) via which streptavidin (SA) can be recruited to selectable sites by the introduction of a biotinylated (B) single strand.

The same group also showed that it is possible to combine the soft lithography technology for surface patterning with polydimethylsiloxane (PDMS) stamps to arrange nanotubes on surfaces [93]. Therefore, they first assembled DNA tubes from a 52-mer oligonucleotide with biotin residues. Then these tubes were aligned on the PDMS stamp and the stamp was dipped in a solution containing streptavidin-conjugated quantum dots. After printing on glass using this stamp the surface patterns obtained were visualized by confocal microscopy using a nucleic acid-binding dye. The images obtained showed a regular pattern of perfectly aligned tubes and quantum dot fluorescence emission only in places where also the stained DNA tubes were detected. The pattern obtained in this process was several hundred micrometers wide. This study beautifully addressed an important issue of nucleic acids in nanotechnology: It is not sufficient to build nanoobjects but in order to be really of use, this world needs to be "connected" to the micrometer world.

Another way of attaching proteins to nucleic acid structures uses covalent protein–nucleic acid conjugates. Towards this concept, Niemeyer et al. have shown that it is possible to form covalent links between proteins and polyamide nucleic acids (PNAs) using expressed protein ligation [94]. These conjugates could then be used for the DNA-directed immobilization—however, so far "only" on DNA arrays (chips). To obtain the conjugates the target protein is expressed as fusion protein with an intein and an affinity purification domain. After immobilization using the latter domain, a treatment with mercaptoethansulfonic acid yielded the C-terminal thioester of the desired protein which could then be coupled to a cystein-terminated PNA. In a more recent study, Niemeyer et al. demonstrated that this technology can be used to generate arrays of live cells [95]: therefore, they again used covalent conjugates of proteins but this time between streptavidin and DNA. These conjugates were incubated with biotinylated RGD-peptides, which are known to be ligands for the integrin surface receptors of, for example, fibroblast cells. Again the system was realized "only" on chips but could again serve as interfacing technology between nucleic acid architectures or scaffolds and living cells.

5. Conclusion

The presented examples clearly show that the nucleic acid nanotechnology has already come quite far. In many seminal studies it has been shown that scaffolds of different topological and mechanical properties—even replicable ones—can be generated. The field has expanded to areas where other interaction principles come into play and this will significantly increase the versatility of nucleic acids as material on the nanometer scale. We have also discussed first applications for nucleic acid nanoarchitectures that have been implemented. While the focus of this review was rather on the side of using nucleic acids to build scaffolds etc., an equally extensive topic is the use of nucleic acids for building devices like motors [96], tweezers [97], thermometers [98], or self-replicating systems [99–101]. Yet another aspect that is beyond the scope of this review is the fact that nucleic acid assembly by Watson–Crick interaction has stimulated an entire field of DNA-based logic [102–104] and even DNA computing [105–108] or DNA cryptology [109]. All in all, nucleic acid technology has really come already very far in the field of nanometer-scaled "intelligent" materials but it is not even close to replacing photolithography as the standard technology. However, it is our firm belief that the molecular bottom-up technology will be the future of synthetic biology and nanotechnology, but we also believe that it is very unlikely that nucleic acids alone will be the solution. Other technologies such as rotaxane-based memory systems have already proven suitable as well [110]. Very much in the same way in which a car or an airplane is not built from just a single material, functional nanoscaled components in, for example, computers or robots of the future will most likely be built from a combination of materials—although maybe not very many. Most importantly, more work needs to be done on interfacing the new nano world with the micrometer world. Given all their power, we think that nucleic acids—especially chemically functionalized nucleic acids—will have their part to play in these developments.

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