Advanced Drug Delivery Reviews 147 (2019) 22–28

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

A minimalist's approach for DNA nanoconstructions

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ARTICLE INFO

Article history:
Received 29 March 2018
Received in revised form 3 January 2019
Accepted 8 February 2019
Available online 12 February 2019

Keywords:
DNA
Self-assembly
DNA nanostructures
DNA nanotechnology

ABSTRACT

Structural DNA nanotechnology takes DNA, a biopolymer, far beyond being the molecule that stores and transmits genetic information in biological systems. DNA has been employed as building blocks for the assembly of designed, nanoscaled, supramolecular DNA architectures for applications in biophysics, structure determination, synthetic biology, diagnostics, and drug delivery. Herein, we review a symmetric approach of tile-based DNA self-assembly. This approach allows the construction of DNA nanostructures from minimal numbers of different types of DNA strands based on sequence and structural symmetries. Some examples of the applications of this approach in siRNA delivery are discussed as well.

Contents

1. Introduction

Nearly 30 years after the discovery of the DNA double helix structure, Nadrian C. Seeman firstly proposed that DNA building blocks containing branch junctions could be used to construct ordered arrays in 1982 [1]. The field of DNA nanotechnology has been emerging since then. DNA is thus from biology but far beyond the molecule that stores and transmits genetic information in biological systems.

The predictable and stable pairs (Watson–Crick base pairing) that form between complementary bases on DNA strands endow DNA with many advantages, in which we highlight the programmability. DNA is structurally stable and DNA double helix is geometrically well-defined. Its compatibility with other molecules allows heterogeneous assembly, which could introduce multiple functions to the resulting nanostructures [2].

Strategies for the fabrication of structures at nanometer-scale include top-down and bottom-up methods. The bottom-up approach to build up nanostructures seeks to have small, simple components to be associated together to form large and complex systems, often through atom-atom integration or molecule-molecule recognition [3–6], which enables systems to be designed such that assembly is programmable and predictable.

Bottom-up fabrication route for the construction of targeted DNA architectures has been increasingly developed over the past three decades. Tile-based DNA self-assembly is the first developed strategy

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https://doi.org/10.1016/j.addr.2019.02.002
0169-409X/

and is an integral part in the evolution of the field of structural DNA nanotechnology [7]. The resulting structures are assembled from a set of short, synthetic, single-stranded DNA strands, which are specifically designed to hybridize with each other at designated segments. These strands first assemble into well-structured structural motifs or tiles, such as double crossover (DX) tiles [8], triple crossover (TX) tiles [9,10], point-star motifs [11–13], and weave tiles [14–17]. They, then, further associate with each other and assemble into large nanostructures, including DNA arrays in one-, two-, and three dimensions (1D, 2D, and 3D), and discrete objects.

In contrast to the tile-based assembly approach, DNA origami [18] and single-stranded tile (SST) approaches [19] construct discrete, large DNA nanostructures from hundreds of DNA strands. These approaches can produce almost any arbitrarily designed DNA nanostructures. In the resulting nanostructures, each part contains unique DNA sequences. While being powerful, these approaches, however, impose experimental complexity and a significant cost limitation, as each design requires the synthesis of hundreds of different DNA strands. In addition, the assembly yields are often low and majority (>80%) of the DNA strands are not incorporated into the final designed structures. Furthermore, in the final structures, only a limited small portion of DNA scaffold is for "working" but most are for structural definition. If the structural regions are repetitive, the number of DNA strands and assembly cost would be greatly lowered [20]. To address these issues, the concept of structure/sequence symmetry of DNA tile motifs has emerged. It allows the construction of nanostructures from minimal numbers of DNA strands [12]. This will result in low cost, good quality control, clear understanding of the cellular impact of each component DNA strands (degradation, toxicity, immunogenicity, *etc.*) when *in vivo* applications are of concern.

DNA provides an extraordinarily versatile material for self-assembly and DNA nanostructures have been demonstrated to be useful in biophysics study [21–25], nanoelectronics [26–28], diagnostics [29], and drug delivery [30-34]. Ideally, a drug delivery carrier should be safe, biocompatible, stable *in vivo*, and capable of targeting diseased area and delivering a high level of drug cargo to this area [35]. In addition, the size and shape of the drug vehicle are believed to play an important role on its biological behaviors [36]. In these aspects, DNAs are unique in their ability to be rationally designed and programmably assembled to size- and shape-defined objects. Uniquely, drug cargos or other agents (*e.g.* targeting agents) can be readily attached to the DNA nanostructures at spatially defined locations.

As discussed below, we will review the symmetric approach for the construction of DNA nanostructures, which allows fabrication of DNA nanostructures from minimal numbers of different types of DNA strands based on sequence symmetry. Then we will briefly discuss such DNA nanostructures for intracellular applications: nucleic acid delivery (siRNA delivery).

2. Sequence symmetry

The basis of construction of DNA nanostructures lies in the connectivity of the biopolymers. In the bottom-up fabrication process to generate nanostructures, DNA self-assembly is achieved through hybridization.

DNA nanostructure design considers primary, geometric structures and nucleotide sequences [37]. There are basically two techniques of sequence design – asymmetric and symmetric approaches. In asymmetric designs, each DNA component strand has a unique sequence and no DNA sequence appears more than once in the entire structure. The final structure could possess geometric symmetry if only DNA backbones are concerned, but no symmetry when DNA sequence is taken into account [12]. Often this approach is precisely stoichiometry-dependent and requires the initial purity control of DNA components involved in order to give the desired DNA nanostructures [8]. To an extreme case, the complex DNA nanostructures from origami and SST routinely require hundreds of DNA component strands with unique sequences.

To decrease the undesired association and potentially avoid the unwanted secondary structures, a principle of sequence-symmetry minimization (SSM) is conducted in the sequence design process [38]. The sequences are selected by minimizing sequences with similarities, which has worked very well for the design of branched molecules. SSM is a general rule when designing a DNA motif.

On the opposite, DNA sequence symmetry approach extensively takes advantage of the structural symmetry. The structural symmetry of the DNA nanostructures dictates the symmetry of DNA sequences. In the symmetric design, it is possible that the same DNA sequence appears multiple times in the final structures when a structural symmetry can relate them with each other. Sequence symmetry could provide many advantages: 1) the number of unique sequences is reduced and the sequence design is simplified; 2) the cost is therefore minimized; 3) the geometric symmetry is ensured to cancel any potential, unpredictable distortions in the assembly.

An example of sequence symmetry design is given for the symmetric four-point-star DNA motif. The four-point-star DNA motif was originally developed by Hao Yan and his colleagues [39]. It consists of nine component strands. Though a clear four-fold rotational symmetry (in terms of DNA backbones) existed in the molecule, all nine DNA strands have unique sequences. Thus, there is no any symmetry for the motif when DNA sequence is taken into account. In 2005, we symmetrized this molecule by sequence symmetry approach to make the DNA sequences to reflect the structural symmetry [12]. The symmetric four-point star motif has a true 4-fold rotational symmetry in terms of both backbone and DNA sequence. It contains only three DNA strands (black, green and red) with unique sequences (Fig. 1). The four red strands are identical to each other and the four cyan strands are identicalas as well. Multiple copies of the identical strands are related with each other by the 4-fold rotational symmetry. Such symmetry allows the motifs to associate with each other isotropically and leads to the formation of DNA 2D arrays with size up to 1 mm, much larger than the arrays

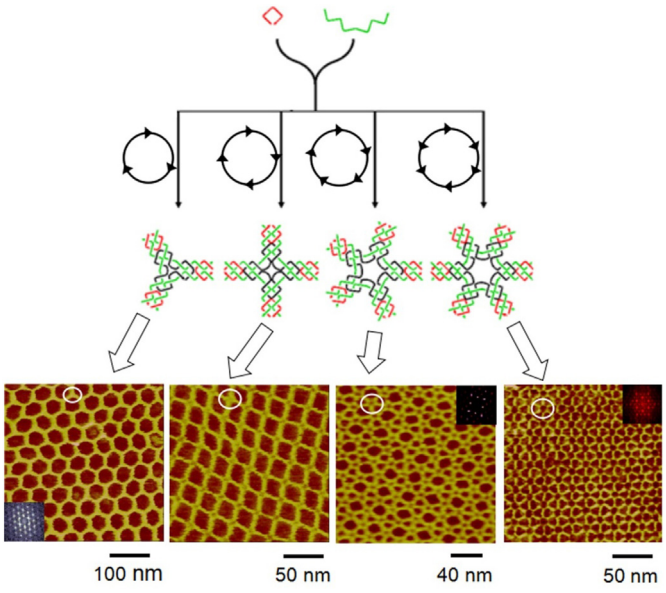

Fig. 1. A family of symmetric star-point motifs: construction and 2D arrays. An n-fold rotational axis runs through the center of an n-point-star motif, which contains one copy of a central n-fold repetitive strand (black) and n copies of two other strands (green and red strands). For different n-point-star motif, the black strand is different, but the green and red strands are identical. On the bottom are AFM images of the 2D arrays assembled from the point-star motifs. White circles highlight the component star-point motifs in the 2D arrays.

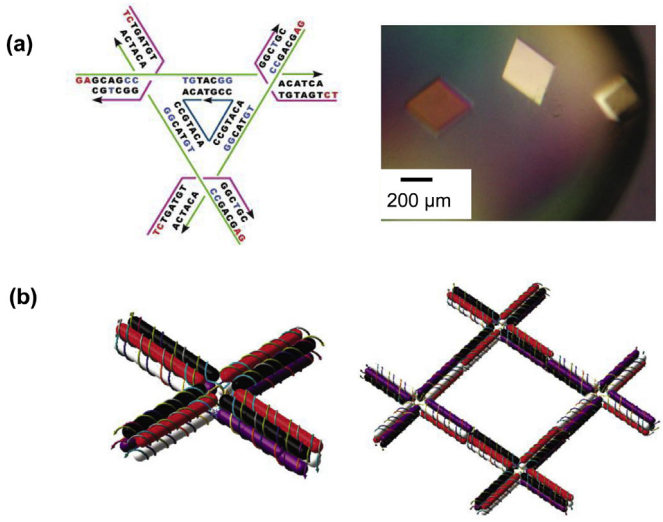

Fig. 2. Non-planar symmetric motifs. (a) A DNA tensegrity triangle. *Left*: Scheme of an individual triangle. It contains one central blue strand, three copies of identical green strands, and three copies of identical magenta strands. *Right*: An optical image of 3D crystals assembled from the tensegrity triangle. (b) Symmetric double-decker tiles. All four arms are identical. *Left*: Sticky-ended double-decker tile; *Right*: 2D lattices formed by double-decker tiles.

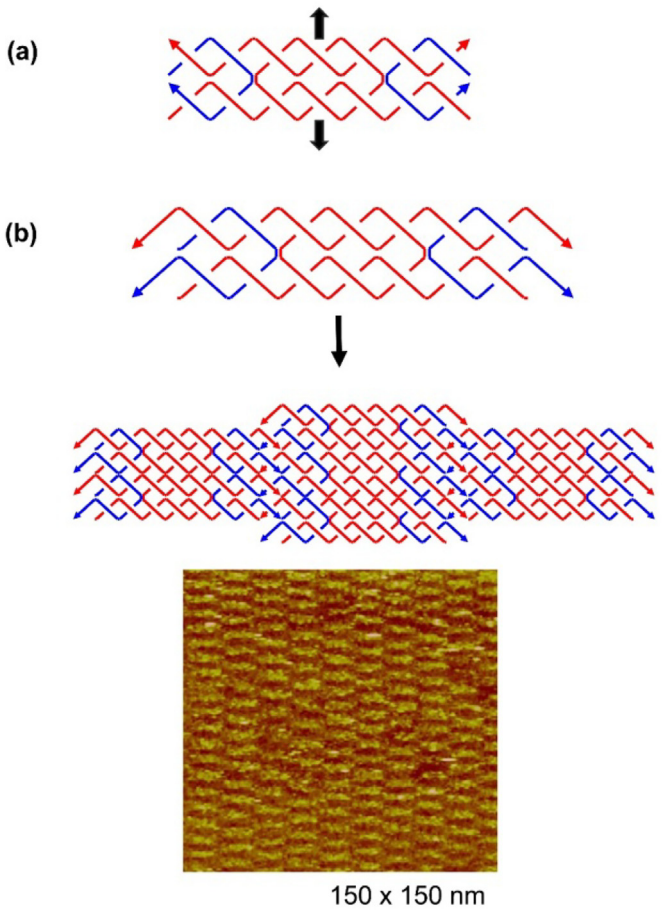

Fig. 3. Symmetric, antiparallel, DNA double-crossover (sDAO) motifs with sequence symmetry. (a) A blunt-ended sDAO molecule. A pair of black arrows indicate a 2-fold rotational axis. (b) A sticky-ended sDAO molecule self-assembles into 2D lattices.

(often ~ 1 μm) made of the asymmetric tiles only on the micrometer scale.

The concept of sequence symmetry dramatically decreases the number of DNA unique strands from nine to three for the four-point-star DNA tile. In addition, this strategy has been experimentally proved to be very successful to construct a family of star-shaped DNA nanomotifs, including three- [11], five- [40], and six-point-star DNA motifs [13], which contain 3-, 5-, and 6-fold rotational symmetries, respectively (Fig. 1). Each of them contains only three different DNA strands and can assemble into robust DNA 2D arrays and discrete 3D DNA nanocages.

Next, we asked a question: can the sequence symmetry strategy be applied to assembly of designed 3D macroscopic crystals? Seeman and we introduced a three-fold rotational symmetry to a rigid, tensegrity triangle motif (Fig. 2a) [41]. Seven strands with three unique sequences constitute the molecule, with three for the crossover (magenta), three extending for the length of each helix (green) and one for the nicked strand at the center (blue). 2 nt-long short sticky ends tailed at the helices cohere to form the well-ordered 3D crystalline lattices.

Later, Thomas LaBean and his coworkers successfully applied sequence symmetry to design a symmetric double-decker tile, in which each arm contains four double helices and four arms are identical to each other (Fig. 2b) [42]. The design simplifies the sequence composition and reduces the number of DNA strands. The tile has been demonstrated to assemble into large 2D and 3D lattices.

3. Homo-polymerization of one component strand

Sequence symmetry-based design minimizes the number of DNA strands and affords a minimalist approach to tile-based DNA nanoconstruction. In the most extreme case, the number of component DNA strands for DNA nanostructures would be only one. Though it is difficult, it is achievable and has been demonstrated in multiple designs including DNA nanotubes [43], 2D crystals [44], 1D and 2D arrays [45], and 3D prisms [46].

To further reduce the number of unique DNA sequences, we introduced palindromic sequences into DNA strands [47]. A palindromic sequence allows DNA strands to self-dimerize. We first applied this concept to simplify the design of a four-stranded, antiparallel, double-crossover (DAO) motif (Fig. 3). Traditionally a DAO motif consists of four different DNA strands though a two-fold rotational symmetry existing in term of DNA backbone. With sequence symmetry, the two red strands and two blue strands are respectively related to each other by the 2-fold symmetry and could be identical. Since the red strands self-dimerize in the symmetric DAO (sDAO), the self-dimerizing domains have to be palindromic sequences. Experimentally, such a motif has been successfully assembled and behaves as a rigid building in following assembly into 2D arrays.

With one step further from the symmetric DAO, the two outside strands can be removed and a motif can be assembled from only one unique strand [43]. As in the symmetric DAO, the strand contains two central domains and each domain is a 16-nt-long palindromic sequence. These two domains allow the strand to self-dimerize into a DX-like motif. When the two domains at 5′ and 3′ ends are also palindromic, the motifs can further hybridize with each other to form 2D arrays. Unlike the traditional DX motif, only one strand crossovers at each junction point. Because of this feature, the resulting 2D arrays are flexible and finally fold into nanotubes (Fig. 4a). In this system, only one unique strand is needed. It contains 52 nts long strand and four palindromic domains, which are 10, 16, 16, 10 nts long, respectively. A similar but smaller system is designed with a 32-nt-long DNA [44]. It contains four palindromic domains (6-, 10-, 10-, 6-nt-long, respectively). Instead of a DX-like intermediate motif, this system involves a long pseudo-continuous DNA duplex intermediate (Fig. 4b).

Motifs other than DX motifs could also be simplified by sequence symmetry approach. One example is the simplification of the T-junction motif [48]. Similar to the 52-nt and 32-nt systems, by introducing palindromic sequences, one DNA strand can homo-dimerize to form a two-stranded motif, in either *Z*- or C-shape determined by the length

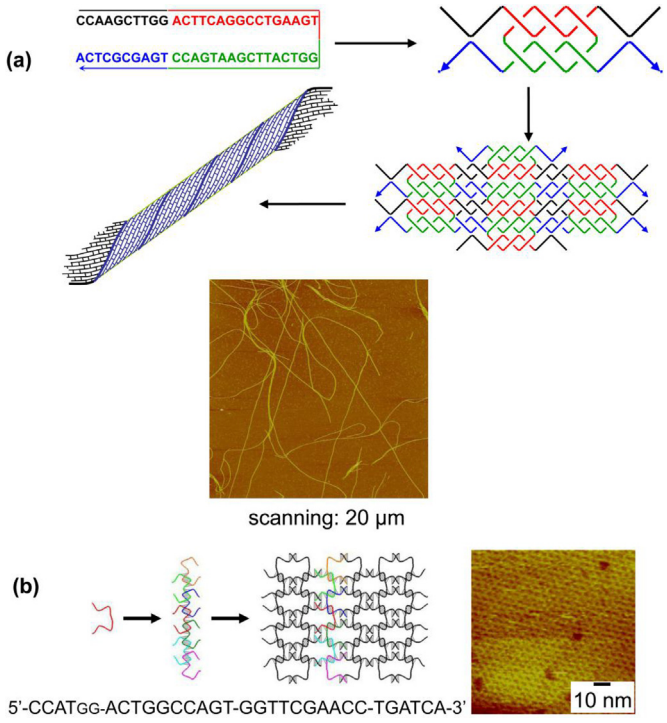

Fig. 4. Assembly of DNA nanostructures from one unique DNA strand with multi-palindromic domains. (a) A 52-nucleotide (nt) strand. The DNA strand contains four palindromic domains (colored different). It first dimerizes into a DX-like motif, which further hybridizes with each other to form 2D structures, and finally folds into nanotubes. (b) A 32-nt system. The strand contains four palindromic domains as circled. *Top*: DNA single strands hybridize with each other to form long duplexes and further assemble into 2D arrays; *Bottom*: DNA sequence.

of central duplex domain. In the motif, a two-fold symmetry axis goes through the center (Fig. 5) [45]. The motif further assembles into 1D or 2D arrays through T-shaped, three-way junction, as determined by the lengths of the two parallel duplex domains.

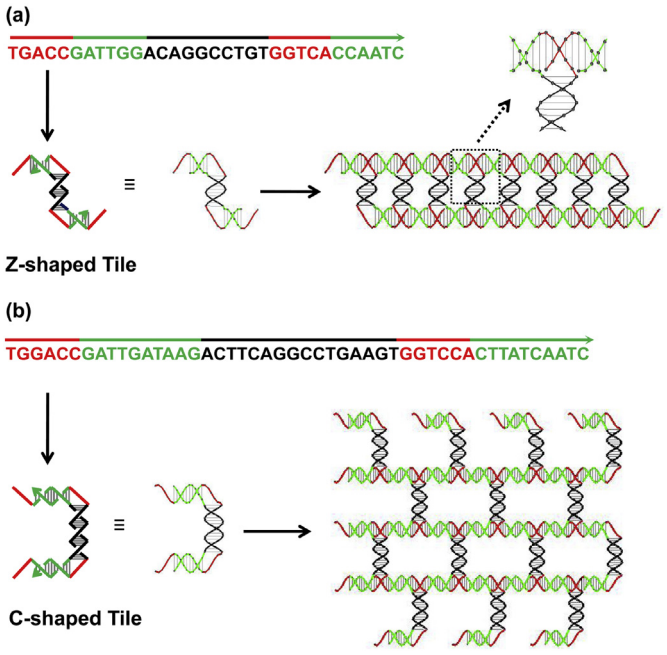

Fig. 5. One DNA strand containing non-palindromic domains homo-polymerizes into nanoarrays. (a) 1D ladders. (b) 2D array. Note that only the black lettered domains are palindromic sequences.

The strategy mentioned above has a potential problem. A palindromic sequence can form either the designed, inter-molecular, homo-dimer or an intra-molecular hairpin byproduct. When the palindrome is short, homo-dimer is preferred. When the palindrome is long, a hairpin is more likely to form. In addition, a unimolecular hairpin is more kinetically favored than the desired two-stranded dimer. To overcome this kinetic problem, we introduced a new inter-molecular interaction: bubble cohesion between interior loops. Bubble cohesion is inter-tile interaction between DNA motifs, in which two bubbled DNA duplexes fuse into one molecule through complementary Watson–Crick basepairing. The interaction does not require free sticky end [49]. In the way, a single strand first quickly folds into a duplex with interior loops. Then the loops hybridize with each other (bubble cohesion) to form two-stranded, homo-dimers. Finally, the homo-dimers further interact with each other *via* T-junctions to form 1D ladders, 2D arrays, and a 3D triangular prisms (Fig. 6). The entire self-assembly is favorable in terms of both thermodynamics and kinetics. The same strategy has also been adapted to assemble an RNA prism [50].

4. Structural symmetry

Symmetry can be applied not only to DNA sequences to simplify the design of individual tiles, but also to the geometry of large DNA nanostructures. One most noticed study is the construction of a series of symmetric DNA nanocages. In this concept, many copies of identical symmetrical motif are associated to form symmetric, large DNA nanostructures. Thus, a limited number of unique DNA strands can hierarchically assemble into complicated DNA nanostructures.

Hao Yan and his colleagues made the first exploration of this approach [51]. They applied symmetry analysis to small, finite, DNA 2D arrays and classified the component motifs into several classes of symmetry-related DNA motifs. All motifs in the same class are identical to each other. For example, a 16-motif, 2D array with C4 symmetry can be divided into $16/4 = 4$ classes. Thus, only 4 types of unique motifs are needed for such a finite array.

We further extended this strategy to construct 3D polyhedra (Fig. 7) [52]. For example, in a tetrahedron, its four, 3-branched vertexes are

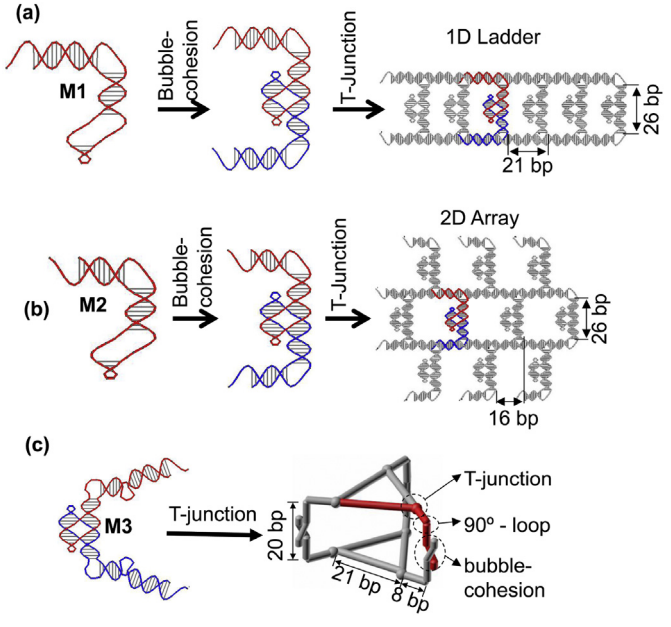

Fig. 6. DNA nanostructures assembled from single component DNA strand that contains no palindromic sequence. Inter-motif interactions are through both T-junction interactions and internal bubble-bubble cohesions. (a) 1D ladders. (b) 2D arrays. (c) A 3D prism.

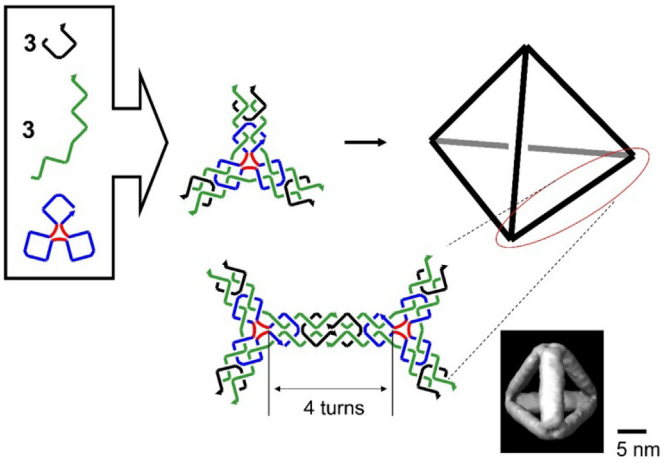

Fig. 7. Self-limited self-assembly of symmetric DNA polyhedra as exemplified by the assembly of a DNA tetrahedron (Only show the assembly of a DNA tetrahedron). Three types of DNA strands assemble into 3-point-star motifs, which further assemble into a DNA tetrahedron. Lower right shows the DNA tetrahedron that is reconstructed from cryoEM imaging.

related to each other by a tetrahedral symmetry. If each vertex is represented by a 3-point-star motif, four copies of identical 3-point-star motifs would assemble into a DNA tetrahedron. The entire DNA nanostructure only requires one type of DNA motif. The overall assemble process resembles the assembly process of a polyhedral viral capsid, which is composed of multiple copies of symmetry-related, identical, capsid proteins. By adjusting the detailed design of the DNA point-star motif (e. g. the number of the branches, the length of the branches, the flexibility of the motifs) and the experimental conditions (e. g. DNA concentrations), a wide range of DNA polyhedra were selectively assembled from only one type of DNA motif, which, in turn, was assembled from only three types of DNA strands. The list of assembled DNA polydedra includes a tetrahedron [52], a cube [53], several prisms [54], an octahedron [55], a dodecahedron [52], an icosahedron [56], and a buckyball [52]. When two types of DNA motifs are combined, the list of assembled DNA nanostructures could be greatly increased [57]. The molecular weight of the assembled DNA nanostructure could reach 5 MD, larger than that of a ribosome, the cellular protein-synthesizing machine.

5. Biomedical applications of the minimalist's DNA nanostructures

Short interfering RNAs (siRNAs) can suppress specific gene expressions; thus, can be used to modulate the expression of genes that are identified as key factors to diseases. However, siRNA molecules usually show poor stability, have short half-life times under physiological conditions, and are incapable of internalizing into cells. Therefore, delivery of siRNAs *in vivo* remains greatly challenging and is crucial for their therapeutic applications. Ideal delivery systems should efficiently and specifically target cells, and protect siRNAs against nuclease degradation during transport [58].

DNA nanostructures are uniquely useful for drug delivery [59,60], not just for safely and effectively loading drug, but enabling drug delivery in new ways [61]. Though many self-assembled DNA nanostructures can work for this purpose, the minimalists' DNA nanostructures assembled from minimal number of component strands have unique advantages compared with asymmetric DNA nanostructures (some of them involve hundreds of different component strands) and bring ease to: 1) synthesizing the component DNA strands and assembling the DNA nanostructures, 2) determining the biocompatibility, toxicity, and immunogenity of each component, 3) requiring minimal amount of work for quality control of each component, 4) understanding the metabolism of each component, 5) minimizing unwanted biological effects of the component strands.

In 2008, we have examined the biocompatibility, toxicity and cellular uptake of self-assembled DNA nanotubes (DNA-NTs). The designed nanotubes showed excellent biocompatibility and low toxicity [62].

In 2015, we loaded the DNA nanotubes (DNA-NTs) with mammalian target of rapamycin (mTOR) siRNA *via* nucleic acid hybridization [63]. The siRNA-DNA-NTs provided a nanosystem for delivery of siRNA to pulmonary arterial smooth muscle cells (PASMCs) under both normal and hypoxic conditions (Fig. 8). Presumably, after the nanosystem was delivered into the cells, the dangling DNA/RNA hybrid duplex was recognized by RNA-induced silencing complex (RISC)-loading complex (RLC). Then the guide RNA was separated from the DNA nanotube carrier and incorporated into an active RNA-induced silencing complex (RISC), which then cleaved the corresponding mRNA [64]. Confocal laser scanning microscopy (CLSM) imaging showed that the siRNA-DNA-NTs were able to penetrate PASMCs *via* a pathway of endocytosis and regulate the autophagy and proliferation of PASMCs.

As delivery vehicles, DNA materials offered the possibilities for precisely positioning siRNAs and fine tuning the vehicle shape in a well-defined manner, thus being able to reach the cellular environment in a predictable behavior. Beside the mTOR siRNA-loaded DNA-NTs, more recently, we showed that a three-stranded siRNA/DNA hybrid as simple as a triangle shape, namely mTOR siRNA-loaded triangular DNA nanoparticles (siRNA-TNPs), could be efficiently transfected into NCI-H292 cells (Fig. 9a) [65]. The DNA endowed the siRNA with increased nuclease resistance while allowing its gene silencing effect of inhibiting the expression of mTOR. The detailed study revealed that the siRNA-TNP uptake was dependent on macropinocytosis and clathrin-mediated endocytosis pathways.

A self-assembled DNA 3-point-star motif was also constructed and loaded with three copies of Rab26 siRNAs (siRab26-DYM) [66]. The complex could efficiently target human pulmonary microvascular endothelial cells (HPMVECs) and silence the expression of Rab26 (Fig. 9b). Interestingly, the siRab26-DYM complex greatly aggravated the LPS-induced cell apoptosis and hyper-permeability of HPMVECs through the promotion of the nuclear translocation of Foxo1, and subsequently activated the signal pathway of Toll-like receptor 4 (TLR4).

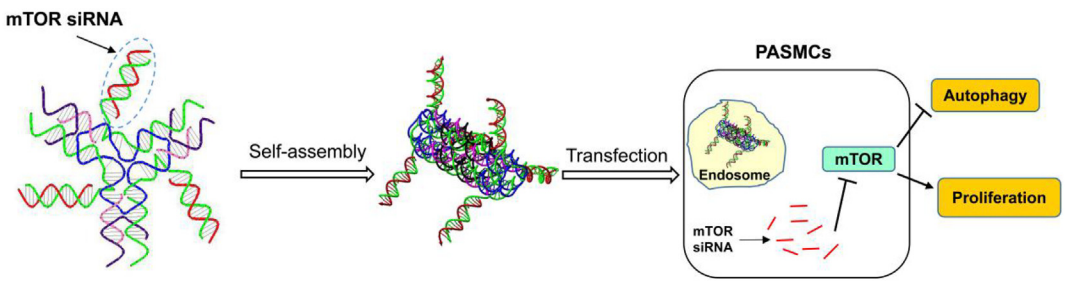

Fig. 8. mTOR siRNA-loaded DNA nanotubes can cross PASMCs membranes *via* endocytosis, and regulate the autophagy and proliferation of PASMCs.

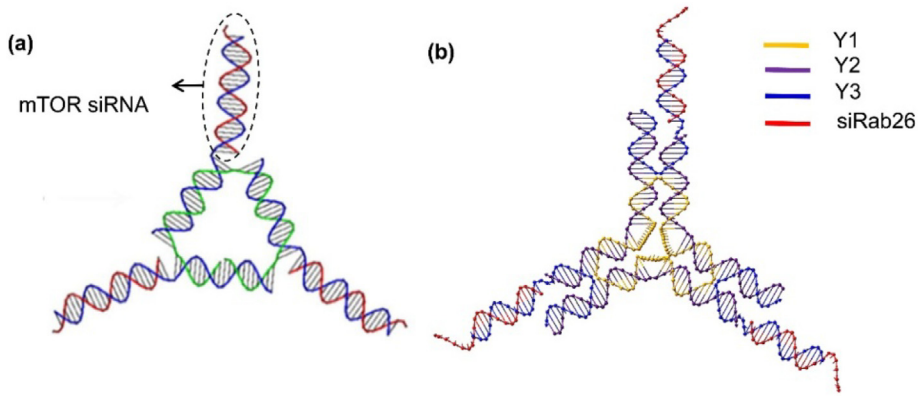

Fig. 9. (a) Scheme of a self-assembled hybrid siRNA/DNA triangle (ssRNA-TNP). Red strands are anti-mTOR guide strands, and blue and dark green strands are DNA strands. Three-fold rotational axis runs through the center of the triangle. (b) Scheme of a self-assembly of symmetric 3-point-star motif that carries three anti-Rab26 siRNAs (siRab26-DYM). One central strand Y1 (yellow), three edge strands Y2 (purple), three peripheral strands Y3 (blue), and three siRab26 (red) are included in the design.

6. Conclusions and outlook

The minimalist's approach of programmed DNA self-assembly is robust and versatile for assembly of a wide range of DNA nanostructures. It takes advantage of (1) sequence symmetry to minimize the number of the required, different, component DNA strands for assembly of individual motifs and (2) structural symmetry to minimize the number of required, different, component DNA tiles for assembly of entire DNA nanostructures. The sequence symmetry strategy helps to fabricate desired DNA nanostructures with minimal DNA strands with low cost, good quality control, clear understanding of the cellular impact of each component DNA strands (degradation, toxicity, immunogenicity, *etc*). However, the symmetry strategy can only generate structures with high structural symmetries and cannot produce asymmetrical species. It can produce only simple or moderately complex structures, such as periodic arrays and polyhedra due to the symmetry requirement. As alternative methods, origami and SST are intrinsically asymmetric.

We would expect that the integration of the symmetry strategy and origami/SST methods will allow the generation of highly complex DNA nanostructures with least number of DNA component strands, to make DNA nanotechnology more practical for intelligent drug delivery, especially in siRNA, antisense oligonucleotide [67], and aptamer delivery [60,68]. These platforms are used for the regulation of gene expression and genetic changes [69]. And they have great potential to facilitate the efficacy of immunoregulation, and work as therapeutic agents for gene therapy in human diseases [70]. Examples include viral diseases, hereditary diseases like myotonic dystrophies (types 1 and 2) and Huntington's disease–like 2, malignancies, and so on [71]. Given the increased understanding of molecular mechanisms of the potential therapeutic roles of siRNAs, antisense oligonucleotides and aptamers on diseases, directly targeting gene/protein is an increasingly compelling therapeutic strategy. However, how to realize the integration of DNA nanostructure-based systems and therapeutic oligonucleotides represents a great challenge to structural DNA nanotechnology, and also a great opportunity. On application side, a major gap in delivery of functional small RNAs (*e.g.* siRNAs, antisense RNAs, and microRNAs) by DNA nanostructures is the lack of *in vivo* study. We envision that, in the near future, significant research efforts will be devoted into this area. Some detailed knowledge about DNA nanostructure-host interactions will be revealed, which will help to develop efficient approaches for *in vivo* delivering oligonucleotide therapeutics.

Acknowledgements

This work was supported by the Fundamental Research Funds for the Central Universities, P. R. China (XDJK2016A015); Key National Science Foundation of China (21535006); and the Venture & Innovation Support Program for Chongqing Overseas Returnees, China; NSFC (81429001); NSF (CMMI–1437301); and ONRN00014-15-1-2707.

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