Self-assembly of large RNA structures: learning from DNA nanotechnology

Jaimie Marie Stewart, Elisa Franco*

Abstract: Nucleic acid nanotechnology offers many methods to build self-assembled structures using RNA and DNA. These scaffolds are valuable in multiple applications, such as sensing, drug delivery and nanofabrication. Although RNA and DNA are similar molecules, they also have unique chemical and structural properties. RNA is generally less stable than DNA, but it folds into a variety of tertiary motifs that can be used to produce complex and functional nanostructures. Another advantage of using RNA over DNA is its ability to be encoded into genes and to be expressed in vivo. Here we review existing approaches for the self-assembly of RNA and DNA nanostructures and specifically methods to assemble large RNA structures. We describe de novo design approaches used in DNA nanotechnology that can be ported to RNA. Lastly, we discuss some of the challenges yet to be solved to build micron-scale, multi stranded RNA scaffolds.

Keywords: RNA, DNA, Nanotechnology, Nanostructures, Self-assembly

1 Introduction

The field of nucleic acid nanotechnology has produced a wealth of self-assembly methods to build nano- and micron-sized structures [1,2]. These biomolecular structures are useful in various applications; in particular they can be used as scaffolds or templates for growth of other materials, and as drug delivery vectors. Both RNA and DNA have been successfully used to build scaffolds with rationally programmable features. RNA structures up to a few hundreds of nanometers large have been demonstrated to assemble in vitro as well as in vivo [2,3]. DNA has however been the polymer of choice to demonstrate 2D and 3D self-assembled structures with size ranging from 20 nm to several microns, due to its stability and the predictability of Watson-Crick base pair interactions [4-6].

Although RNA and DNA share many general features, they also present many unique chemical and structural properties; Figure 1 summarizes some of the structural characteristics of DNA and RNA. These differences have prompted the development of distinct approaches to programmed self-assembly. Specifically, in silico sequence design to satisfy domain complementarity requirements combined with Holliday junction motifs dominates the field of DNA self-assembly [1]. In contrast, the exploitation of conserved, naturally evolved motifs with predictable tertiary structure (such as kissing loops) dominates RNA self-assembly methods [8].

This brief review provides a comparison of existing approaches to the design of large, multi-component RNA and DNA nanostructures. For the reader’s convenience, an abridged list of design methods and protocols is reported in Tables 1 and 2. We focus in particular on the problem of building large, multi-stranded RNA scaffolds with the potential of being stable or assembling in vivo. The construction of large RNA nanostructures presents significant challenges relative to small RNA nanoparticles [2], because of the higher likelihood of RNA strands to form undesired secondary structures. We suggest that methods developed for DNA tile systems may be viable to build large RNA assemblies which could be expressed and assemble in vivo; we highlight advantages, limitations, and challenges of this strategy.
2 DNA nanotechnology relies on in silico sequence design where individual strands have minimal secondary structure

The early objective of DNA nanotechnology aimed to form robust three-dimensional crystals to address the “crystallization problem” [9]. Since then, several other breakthroughs have been accomplished such as the assembly of large lattices [4,10], 2D and 3D structures [5,11,12], and nanomachines [13-15]. It is important to note that the scope of DNA nanotechnology goes beyond materials science and biomedical applications. Adleman’s Hamiltonian Path experiment [16] demonstrated that DNA, a biological programmable molecule, could be used as a tool to solve a computational problem; Winfree’s DNA tiles were designed to build algorithmic assemblies [4,17,18].

The design of DNA self-assembling systems generally starts with the identification of complementary domains that form double helices connected via Holliday-type junctions and achieve the desired spatial features when the participating strands bind. After domain-level interactions are specified, sequences of bases can be optimized to maximize the probability of forming the target complexes by free energy and energy strain minimization [19]. The thermodynamic parameters of Watson-Crick base pairing are well characterized [20]; the structure of the DNA double helix is also very well understood (Figure 1) as well as the Holliday junction motif widely used in DNA nanotechnology [21]. Thus, sequence-specific energetics and geometry of a target DNA assembly can be easily modeled for the purpose of sequence optimization, which can be done with a variety of software toolboxes [22-24]. This two-stage design relies on the fact that a very large space of sequences can be identified to satisfy desired domain-level interaction constraints.

There are two main approaches to design DNA nanostructures: the tiling method and the origami method. Table 1 summarizes the features of these two approaches. Nadrian Seeman originally studied DNA tiles [25] as a model for double-strand breaks in DNA recombination processes. The primary element in DNA

![Figure 1. Comparison between DNA and RNA double helices. Image generated using Chimera [7].](image-url)
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Tiles is the crossover junction, which can be defined geometrically by determining the positions where the phosphodiester linkage crosses over from helix to helix [21]. Seeman’s tile monomers consist of multiple short DNA strands (20–60 bases) assembling into two double helices rigidly connected by two junctions, which create a double crossover (DX). Various types of DX tiles were later modified to include single-stranded “sticky end” domains, and operate as monomers whose binding affinity could be specified by sticky end complementarity [4] (Figure 2A-C). Sticky ends must not over or under twist the DNA double helix or negatively affect the stability of the target structure. DX tiles have been used to build two-dimensional lattices and three-dimensional tubular structures that can assemble to micron-scale size. Several different tile designs have been demonstrated and characterized, ensuing in different characteristics such as rigidity, bend, and stability of tiles, and affecting the tile assembly shape [11,26-28]. In general, DX tile-based DNA systems function as a programmable “jigsaw” puzzle that grows in a scalable manner and can implement algorithmic assemblies; however with a finite number of tiles only simple, repeated patterns and structures (such as nanotubes, ribbons, and lattices) can be generated.

The DNA origami method addresses the limited pattern complexity that can be obtained with tiling systems. This method uses a long single-stranded DNA which folds into a target pattern by short DNA strands that, each creating a junction, act as “staples” to stabilize the structure (Figure 2D). The original method of designing DNA origami consisted of creating a structural outline using the M13 bacteriophage sequence, and then producing staple sequences to assist in structural folding. Different software packages to automate origami design have been developed, such as Tiamat [34], SARSE [35], and caDNAno [36]. This method of assembly offers a higher degree of spatial control over self-assembly in comparison to tile-based systems [12,29,37,38] (Figure 2E and F). Furthermore, DNA origami structures have been demonstrated to offer positional control down to the atomic level [33] (Figure 2F-M). However, the complexity of an individual origami limits the scalability of its size relative to tile-based systems, which can assemble several microns in length.

Table 1: Summary of the most common design approaches to build DNA and RNA nanostructures. List of abbreviations: MS, multi-stranded; SS, single-stranded; DS, double stranded; SST, single-stranded tile.

<table>
<thead>
<tr>
<th>DNA</th>
<th>RNA</th>
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<tbody>
<tr>
<td><strong>Method</strong></td>
<td><strong>Tiles</strong></td>
</tr>
<tr>
<td>Components</td>
<td>MS or SS Short strands (20–60 bases) In silico design Arbitrary sequence design space</td>
</tr>
<tr>
<td>Structural features</td>
<td>Holliday junctions SS/DS domains Sticky ends</td>
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Recent work has tried to address this limitation with the development of origami tiling or connector systems. Individual origami tiles or blocks can be interfaced in two ways: the first approach is the use of sticky ends [39,40,41], where binding patterns are assigned by Watson-Crick base pairing. The main disadvantages of this approach are the high yield of imperfect structures and the required long annealing process; the method is also sensitive to the annealing parameters (high temperatures yield defective tiles that cannot interact, and low temperatures result in formation of crystal nuclei and aggregation). The second approach is to use stacking interactions between blunt ends at the edges of individual origami [42]. Stacking interactions have been particularly useful to generate dynamically reconfigurable bonds in 3D origami [43] (Figure 2F). The main challenge of this approach is that poor models make it difficult to energetically distinguish correct and incorrect bonds by design [42].

The complexity achievable by DNA tile systems has been recently expanded by single-stranded tiles (SST) [31]. Each SST interacts with other tiles via four distinct domains generating a single crossover junction (Fig. 2G). This motif can be used to build large, programmable, and scalable ribbons and lattices [31] (Figure 2H), but also 2D and 3D shapes [44,45,32] with complexity comparable to that of DNA origami (Figure 2 D-J). Here, short unique strands each having a role akin to that of a pixel or a voxel, yield objects with sizes ranging in the order of few hundreds of nanometers. This approach does not require a long DNA scaffold, which can present a significant design constraint and pose re-design challenges. However, the size versus complexity tradeoff is not yet solved: complex
shapes require thousands of individually designed SSTs with uniquely interacting domains, a requirement that can limit their scalability.

Complex structures have been recently built using wireframe approaches: grids of double helical domains are connected via multi-arm junctions to generate 3D structures. Relative to DNA origami, these structures are less tightly packed and, as a consequence, have the potential to be more stable in physiological conditions [46-49]. Systematic topological design of these structures is challenging and can represent an obstacle to their size scalability. Finally, the largest existing DNA nanostructures have been built using a “tensegrity triangle” motif [50,51], which generates three-dimensional crystals that can assemble scalably up to hundreds of micrometers in size. These structures rely on sticky end domains to connect the four-armed junctions of the tensegrity triangles; they can be useful as rigid scaffolds for other ligands, or as biocatalysis vehicles [52].

3 RNA nanostructure design largely relies on single-stranded building blocks with stable tertiary structures

RNA is an ideal material for biomedical applications. RNA is produced by cells, it self-assembles, it is stable at low pH, and it can be a functional molecule as showcased by RNA nanoparticles such as ribozymes, aptamers and siRNA [53,54]. By combining functional domains and domains programmed for assembly, RNA nanostructures can control the spatial arrangement of functional molecules [2,55,56]. RNA nanotechnology is an emerging field with

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**Figure 3.** A: Schematic and TEM of RNA filaments assembled from tecto RNA molecules, image reproduced with permission from [68], 2006, Oxford University Press. B: Schematic of RNA anti-prism and cryo-EM of anti-prism particles, image reproduced with permission from [69], 2010, NPG. C: Single-stranded RNA tile based on DNA DAO tile and AFM image of hexagonal lattice assembly, image reproduced with permission from [58], 2014, AAAS. D: Schematic of tecto-square and AFM image of tecto-squares, image reproduced with permission from [57], 2004, AAAS. E: Schematic of hexagonal RNA nanoring based on kissing loops and AFM images of clustered nanorings, image reproduced with permission from [70], 2011, ACS. F: Schematic of RNA nanoring functionalized with dsRNA, cryo-EM image of nanorings functionalized with dsRNA, and class averages of of nanorings observed by cryo-EM, image reproduced with permission from [55], 2014, ACS.
recent demonstrations of structural assembly of large RNA arrays [57], large tiles [58], and small 3D structures such as cubes and rings [59]; functionalized structures were utilized successfully as vectors for cancer and HIV therapies [55,60,61] (Figure 3).

The self-assembly pathways of RNA include Watson-Crick base pairing non-canonical base pairing (for instance, G-U wobble pairs); stacking interactions are also stronger than in DNA. RNA A-form helices are more compressed and rigid [62,63] as well as more thermodynamically stable than DNA B-form helices [64,65]. Non-canonical base pairing can drastically increase the stiffness and thermodynamic stability of RNA structures [66]. This increased thermodynamic stability is a major reason why it is difficult to design and assemble large RNA nanostructures.

Non-canonical base pairing characterizes many evolutionarily conserved tertiary structure motifs, in particular kissing loops, which are not found in DNA. Established methods for RNA nanostructure design heavily rely on natural RNA motifs to produce complex and functional nanostructures [67]. These motifs have non-arbitrary sequences and generally rely on folding of relatively short, single-stranded elements. HIV kissing loops are the most heavily used motif to build large RNA assemblies.

Single-stranded RNA molecules with specific tertiary structure are building blocks, or tecto-RNAs, in “RNA architectonics”, one of the most established methods for RNA nanostructure design [71]. Tecto-RNA elements with desired 3D geometric interactions are identified with NMR or X-ray crystallography. Then, 3D structures are mapped into secondary structures and into a finite number of single-stranded RNA sequences that yield the desired RNA motif. The direction, spacing and angle at which tecto-RNA elements interact can be determined from the known crystal structure of the sequences [72]. Tecto elements can be identified starting from published structures or databases; RNAJunction, for instance, provides a list of junctions, kissing loops, internal loops and bulges selected from PDB coordinate files [73]. Softwares such as NanoTiler [74] can be used to compose motifs to fulfill topological specifications and build larger structures. Examples of RNA architectonics include filaments [68] (Figure 3A), polyhedra [69] (Figure 3B) and jigsaw puzzle pieces or tectosquares [57] (Figure 3D and E). Like DNA tiling systems, RNA architectonics relies on interactions among relatively short molecules (30–60 bases); however, the salient feature of individual tecto-RNAs is their well-defined secondary and tertiary structure, while DNA tile individual strands are typically designed to present no secondary structure (as isolated strands) and exploit inter-strand Watson-Crick bonds.

The inclusion of long RNA strands in nanostructures poses one major challenge: RNA sequences are likely to present a large number of local folding traps, which can hinder the formation of the desired assembly. This issue has been recently addressed in a single-stranded RNA origami tile approach [58], which actively exploits local folding of long strands; tiles of variable size (from 2 helix DX tiles to 6 helix tiles) were demonstrated and successfully assembled in larger structures (Figure 3C). These single-stranded tiles were designed from the DNA-AO and DNA-AE tile geometries, where domain lengths were adapted to

| Table 2: Summary of protocols used to produce DNA and RNA nanostructures (abridged). List of abbreviations: TA, Tris Acetate; TE, Tris EDTA; TAE, Tris Acetate EDTA; TB, Tris Borate; TBE, Tris Borate EDTA; TMS, Tris Magnesium Saline; RT, room temperature. |
| DNA | RNA |
| Method | Tiles | Origami | Tecto-RNA | De novo design | Co-transcription |
| Buffer conditions | TAE; Mg"⁺ [11] or Na"⁺ | TAE or TE; Mg"⁺ or Na"⁺ | TBE; Mg"⁺ and/or Na"⁺ | Snap cooling, 95°C to 4°C, then incubation at 30°C prior to gel extraction. For tectosquares assembly cool 50°C to 4°C [57,70] | Transcription buffer, NTPs with Mg"⁺, Na"⁺, K"⁺ [61,58] |
| Thermal treatment | From > 90°C to RT [11,26] | 95°C to 20°C [5], 95°C to 4°C [29] | Snap cooling, 95°C to 4°C, then incubation at 30°C [59,58]. Slow cooling from 80°C to 4°C [96] | Snap cooling less than Less than 1 hour [59]; 10 minutes [58] to 4 hours [61] |
| Assembly time | Individual tiles: 5 minutes to 2 hours | For single layered objects: less than 2 hours [5,29]; for multi layered objects: several days [12] | Snap cooling less than Less than 1 hour [59]; 10 minutes [58] to 4 hours [61] | 16 hours, mica-assisted [57,70] | Snap cooling from 40°C to 30°C [58] |
A-form helices (Figure 1). Sticky ends are substituted with kissing loop domains and crossover domains are replaced with kissing loop interactions at a 180 degree angle. In terms of assembly size, RNA origami tiles are expected to grow in a scalable manner like DNA tile-based systems; the largest lattice size reported in [58] measures roughly 500 nm. These structures are transcribed and folded in a one-pot reaction, and their assembly is mica-assisted. These structures have the potential to be successfully expressed in vivo.

Because RNA can be encoded into genes and transcribed inside cells, isothermally assembling RNA nanostructures could be produced in vivo and function as programmable scaffolds. Tecto-RNA based structures may be difficult to produce in vivo; protocols used for their assembly require several annealing and purification steps (Table 2). Tertiary structures may not fold as predicted without annealing and in the absence of appropriate ionic conditions; their misfolding may lead to low yield and unwanted interactions between the RNA nanostructure and cellular components. It may be possible to circumvent these limitations by using de novo design methods akin to those used in DNA nanotechnology.

4 De novo design methods from DNA nanotechnology can be adapted to RNA

As outlined in the previous sections, established RNA nanostructure design approaches vastly rely on conserved, single-stranded sequences folding into known secondary/tertiary structures that yield inter-molecular interactions of predictable geometry. In contrast, DNA nanostructure design methods are largely based on de novo design and in silico optimization of an ensemble of sequences, where individual strands are generally required to have minimal secondary structure; multi-stranded double helical

Figure 4. A: Schematic of multi-stranded RNA cube and TEM images and reconstruction of RNA cubes, image reproduced with permission from [59], 2010, NPG. B: Schematic of tetramer tile, Cryo-EM and reconstructed images of RNA nanopris, image reproduced with permission from [77], 2015, NPG. C: Schematic of multi-stranded DNA/RNA hybrid tiles that assemble into lattices and AFM images of hybrid DNA/RNA tubular and lattice structures, image reproduced with permission from [78], 2010, NPG. D: Schematic of RNA tile formation and AFM images of filaments and lattices assembled in vitro and used for in vivo scaffolding, image reproduced with permission from [3], 2011, AAAS.
domains are connected via suitable junctions and sticky ends. In principle, this approach could be ported to RNA nanostructure design and yield RNA nanostructures with complexity comparable to that of DNA nanostructures [1]. Additionally, double helical binding domains, in contrast to kissing-loop motifs, may be immediately interfaced with a variety of nucleic acid dynamic circuits based on strand displacement reactions [75,76].

Afonin and coauthors demonstrated de novo designed 3D RNA scaffolds [59], where target structures were designed using NanoTiler [74] and Accelrys Discovery Studio [79] to produce 3D computer models; sequences were identified using Monte Carlo optimization algorithms. These structures were demonstrated to assemble co-transcriptionally, showing their potential for in vivo expression (Figure 4A). Recently, the assembly of a rationally designed de novo RNA nanoprism was achieved using the DNA T-junction motif [77]. The nanoprism formed from “S-shaped” double stranded monomers that include two T-junction motifs. One T-junction motifs causes the monomers to form a tetramer and the other causes tetramers to dimerize and form a prism (Figure 4B). T-junction motifs rely on binding of sticky ends to a single-stranded loop. These structures are capable of co-transcriptional assembly as Afonin’s nanocubes; however, in both cases abortive transcripts are prevalent and results in a lower target structural yield and unwanted assemblies.

Recently, RNA nanostructures were built using directly the DNA origami approach [80]. A helix bundled tile and helix bundled tube were designed with a long RNA scaffold strand folded into the desired structure by small RNA staple. Assemblies were also obtained with chemically modified RNA scaffolds. This RNA origami has a limited size of approximately 40 nm, which may be difficult to scale up due to possible kinetic traps in the scaffold strand. As noted earlier, the origami method appears to be less scalable than tile-based assemblies that produce nanometer to micron-sized structures.

A hybrid DNA/RNA DX tile system, where sequences are optimized in silico using Sequin [24] was demonstrated in [78]. These tiles assemble in tubular or ribbon-like structures that can grow up to several microns in length (Figure 4C). Because DNA/RNA helices fold in A-form as RNA-RNA helices (Figure 1), it is reasonable to expect that the same DX tiles could be built using solely RNA strands. These RNA tiles could generate large, scalable semi-rigid assemblies and demonstrate that tiling methods for DNA nanostructure design can be ported to RNA.

A single crossover tile with the potential to assemble into 1D and 2D scaffolds in vivo was proposed in [3]. Tiles were designed from sequence symmetric RNA monomers, which bind to form dimers; polymerization of dimers can occur through sticky ends (Figure 4D). Polymerization and dimerization domains were designed to generate a reaction funnel, so that dimerization occurs first, followed by polymerization: sticky ends are protected by hairpins and become exposed only after dimerization. Self-assembly is achieved via formation of double helical domains and Watson-Crick base pairing. These structures incorporate hairpin loops to localize cellular components, and were used to increase the yield of a hydrogen producing pathway in bacteria. The simplicity and hierarchical assembly of these tiles suggests that this design is well conceived for co-transcriptional isothermal assembly. AFM images provided in [3] indicate the assembly of structures with sizes in the range of 200–300 nm, however the structure of individual tiles or the yield of correct assemblies is unclear (Figure 4D). Recently, an RNA DAO-O tile motif [11] was used to successfully co-localize enzymes in vivo [81], but features and yield of the assemblies are yet to be identified.

5 Challenges and outlook

An exciting research direction in the field of RNA nanotechnology is the development of large assemblies in vivo. Artificial RNA scaffolds in cells could serve functional purposes and localize molecular components, as recently suggested [3,81]; it may be even possible to use cells as factories to produce and expel RNA scaffolds for other applications. In vivo assembled structures should form isothermally without a compromise in yield, and should not be toxic to cells or tissues, as well as having a functional role. We identify two critical research areas that present several challenges: 1) Predictive design software is needed to identify feasible and simple structural designs with high likelihood of yielding correct assemblies. Because tectonic elements may be difficult to fold, a possible route could follow the DNA nanotechnology approach: multi- or single-stranded RNA structures could be designed by minimizing the secondary structure of individual strands, while maximizing the binding probability of double stranded domains, relying on Holliday junction structural rules. 2) Assembly methods should be a one-pot, isothermal process with a focus on co-transcriptional assembly in biocompatible conditions; in contrast, the majority of existing RNA structures require specific annealing protocols (Table 2). Co-transcriptional methods should primarily produce the correct product with minimum abortive and elongated transcripts.
There are several nucleic acid software packages that support nucleic acid nanostructure design; the majority focus on DNA [22,24,36,97]; very few of these programs can be used for both DNA and RNA, and even fewer programs are geared directly for RNA [98,99]. The most used programs for RNA nanostructure design are NanoTiler [74] and RNA2D3D [100]. NanoTiler is used to optimize motif arrangements and helical spacers, given a target topology; motifs can be identified from the RNAJunction database [73]. NanoTiler is also capable of sequence optimization. NanoFolder is an online utility for sequence design and secondary structure prediction of multi-stranded RNA systems, which can handle pseudoknots [101]. As NanoFolder, NUPACK can optimize RNA sequences needed to produce simple multi-stranded target secondary structures, with single or multi-state targets, and with concentration specifications; however, NUPACK does not handle pseudoknots [22] and it is mostly targeted to reaction pathway design. Both NanoTiler and NUPACK do not offer 3D visualization of structures. Based on primary and secondary structure information, RNA2D3D generates a 3D model. The model and/or the user-defined sections can undergo energy minimization and short molecular dynamics simulations; however, this software is not intended as a tool for 3D visualization of de novo designs. It is worth remarking that, to our knowledge, none these softwares can handle ionic conditions that deviate from standard scenarios. The availability of integrated toolboxes, with features comparable to DNA toolboxes (such as Tiamat [34], SARSE [35], or caDNAno [36]), will enable the automated construction of capable large scale RNA structures and vastly expand the range of achievable RNA structures.

Co-transcriptional assembly eliminates the time-consuming laborious steps involved with the extraction and purification of RNA. Furthermore, it is the first step toward expressing the desired RNA nanostructures in vivo. However, undesired transcriptional products commonly encountered in vitro suggest that it will be challenging to obtain in vivo self-assembly with high yield and low error rates. Two most undesirable phenomena are the production of a) large amounts of abortive transcripts and b) elongated (run-off) products; these phenomena are frequently observed in bacteriophage polymerase-based in vitro transcription, although they have recently been measured in vivo as well [105]. Abortive transcripts are the result of the relative instability of the transcription complex in the initial stages of transcription [102-104]; these transcripts can be 2–13 bases long and may form stable complexes competing with the desired assemblies. Abortive transcription is sequence dependent, and can be mitigated using G-rich transcription initiation sequences [102]. Elongated or “run-off” transcription is a well-known phenomenon when using linear templates for transcription [106]; incorrect products with up to twice as long as the desired transcript can reach 70% of the total synthesized RNA. This phenomenon appears to be a self-coded process, and it can be reduced by enforcing strong secondary structure at the 3’ end of the transcript [106,107]. Unfortunately, the imposition of specific sequence content or secondary structure to ensure correct yield of products can limit the nanostructure sequence design space.

Despite several technical challenges are yet to be solved, the field of structural RNA nanotechnology has demonstrated a variety of large assemblies. We suggest that an exciting long-term research direction for the field is the construction of responsive, dynamic RNA structures. Dynamic control of RNA assembly would yield spatial and temporal control over self-organizing RNA molecules. Applications would include, for instance, more efficient RNA-mediated drug delivery methods, where functional RNAs may be designed as a “passenger” on a large structure that can only be released upon recognition of a temporal signal and reconfiguration of the structure itself. Another fundamental goal would be that of producing dynamically controllable in vivo assemblies to localize cellular components both spatially and temporally, mimicking the function of cytoskeletal scaffolds. RNA structures in vivo could respond to transcription factors that control their production or to physical stimuli via aptamers.

We believe that de novo nanostructures built with a single or multi-stranded tile system would serve as an ideal candidate for dynamic reconfiguration in comparison to origami assembly. DNA tile topologies would be easily adaptable to RNA with suitable adjustments that account for their different geometries (Figure 1). DNA single-stranded tiles (SST), for instance, could serve as a starting point to build RNA SSTs assembling into tubes or sheets with scalable dimension. DNA SSTs can assemble isothermally in biocompatible conditions [108], which suggests that their RNA analog could have similar properties. While reconfiguration of origami structures is possible, it is limited by the large number of specific, semi-rigid interactions between the staple and scaffold strands dynamic [109]. In contrast, tile systems assemble scalably and thus they can disassociate and reconfigure easily. Assembly, disassembly, and reconfiguration of structures may be controllable via toehold mediated branch migration [110,75,111]. This approach has been used, for instance, to switch the conformation of a 2D DNA lattice [112]; assembly of DNA nanotubes has been triggered with a complex catalyst DNA.
circuit [76], demonstrating that two different classes of nucleic acid devices (dynamic circuits and nanostructures) can operate synergistically. It is unclear whether strand displacement networks could be easily interfaced with RNA nanostructures built from tectonic motifs, such as the large RNA origami tiles demonstrated in [58]; to our knowledge, displacement or strand invasion of tertiary structures such as kissing loops has not been investigated.

RNA is a programmable nanomaterial that can generate large biologically functional structures. Design methods borrowed from DNA nanotechnology have the potential to expand further the complexity of achievable RNA nanostructures. In particular, tile systems akin to those typically used in DNA nanotechnology could yield very large RNA nanostructures, which could find application as delivery vectors for multiple RNA nanoparticles. Large in vivo RNA structures could serve as programmable scaffolds to spatially control or compartmentalize other cellular components. Dynamic control of these large structures could be achieved by directing tile assembly with cellular or environmental signals, and would provide a further programmable layer of control for cellular processes.

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