High-order structures from nucleic acids for biomedical applications

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Over the past 40 years, research in the fields of DNA nanotechnology and RNA nanotechnology has taken nucleic acid molecules out of their biological contexts and harnessed their unique base-pairing and self-assembly properties to generate well-defined, organized, and functional supramolecular architectures. Capitalizing on an intrinsic biocompatibility and the ability to tailor size, shape, and functionality from the bottom up, recent work has positioned high-order nucleic acid structures as powerful biomedical tools. This review summarizes advances in nanotechnology that have enabled the fabrication of synthetic nucleic acid structures. Nucleic acid-based platforms for biosensing and therapeutic drug delivery are highlighted. Finally, an outlook that considers the limitations and future challenges for this field is presented.

Advances in DNA nanotechnology

DNA junctions

Deoxyribonucleic acid (DNA) is well known as the macromolecule that encodes genetic information. Taken out of its biological context, DNA is also an attractive material for bottom-up
fabrication. First, the composition of DNA is known. DNA sequences are made up of four nucleotides: adenine, cytosine, guanine, and thymine (A, C, G, and T, respectively). Second, DNA participates in some of the most predictable interactions of any natural or synthetic molecule. Indeed, DNA sequences form hydrogen bonds according to Watson–Crick base pairing rules (i.e., C pairs with G and A pairs with T), and these interactions confer on DNA the capacity for precise molecular recognition and programmable self-assembly. Finally, the structure of DNA is defined at the nanometer (nm) scale: DNA helices adopt B-form geometry, with 10.5 base pairs (bp) per turn, a diameter of 2 nm, a helical pitch of 3.4 nm, and a persistence length of 50 nm. However, in nature, DNA exists predominantly as a duplex with a linear helical axis, which is poorly suited for fabrication in three dimensions (3D).

In 1982, Nadrian Seeman conceived of using branched DNA molecules, or junctions, to assemble DNA structures in 2- and 3D. Seeman’s inspiration was the Holliday structure, a mobile intermediate of genetic recombination that consists of a single strand exchange, or crossover, between two DNA duplexes. In a seminal publication, Seeman proposed rules for constructing immobile DNA junctions from multiple strands and suggested fitting the junctions with ‘sticky ends’. Sticky ends are single-stranded overhangs, and cohesion between sticky ends in DNA generates a helix with standard B-form local geometry. By programming DNA junctions to self-assemble via sticky-ended cohesion (Fig. 1A), Seeman imagined the creation of extended DNA arrays, including 2D lattices and 3D crystals. Seeman’s vision was that DNA crystals with embedded recognition motifs could be used as hosts to organize proteins and other macromolecule guests for structure determination by X-ray crystallography (Fig. 1B). In the years following Seeman’s innovative proposal, a variety of DNA structures were created, including multi-way junctions, geometric shapes, knots, and polyhedra, and thus the field of structural DNA nanotechnology was established.

DNA tiles

While early work in the field of DNA nanotechnology demonstrated that target topologies could be generated by manipulating flexible junctions, the creation of specific 2- and 3D geometries hinged on the development of more rigid motifs. To this end, a variety of DNA ‘tiles’ with high structural integrity have been developed. One notable example is the double-crossover (DX) tile. In contrast to the Holliday junction, which features one crossover between two DNA duplexes, the DX tile features two crossovers (Fig. 2A). Accordingly, the DX tile has a stiffness that is twice that of linear, double-stranded DNA. DX tiles with sticky ends have been shown to self-assemble into periodic and aperiodic 2D lattices (Fig. 2A). Further research has produced triple crossover (TX) and paranemic crossover (PX) tiles, multi-point stars, double-decker tiles, T-junctions, and other tiles for assembling DNA lattices of varying patterns and periodicities, 3D DNA objects with controlled sizes, and even DNA-based nano-mechanical devices.

Additionally, in 2009, Seeman’s group used a tile known as the tensegrity triangle to produce the first rationally designed, self-assembled DNA crystal. In the tensegrity triangle, seven oligonucleotide strands come together to form a structure comprising three struts and three four-way junction vertices, while two-nucleotide sticky ends mediate self-assembly in 3D.
The development of self-assembling DNA crystals constituted an important step toward realizing Seeman’s aforementioned vision for the field of DNA nanotechnology. However, the crystals diffraction to only 4 Å resolution.33 Similarly, self-assembling DNA crystals designed to contain two tensegrity triangles per asymmetric unit diffraction to only 5 Å resolution.34 In recent years, improvements to resolution and crystal stability have been made by incorporating 5’ terminal phosphates into certain component DNA strands,35 by changing the length36 and composition37 of the sticky ends, and by introducing additional stabilizing DNA strands,38 crosslinks,39 and covalent bonds.40 However, further improvements to resolution will be necessary in order to maximize the utility of DNA crystals as tools for macromolecular structure determination by X-ray crystallography.

DNA origami

In a 2003 publication, Yan et al. described the creation of DNA lattices from DX tiles self-assembled around long (~300 nucleotide; nt) strands of DNA.20 The following year, Shih et al. reported a DNA octahedron self-assembled from a 1.7 kilobase (kb) strand of DNA and five 40-nt strands.42 Inspired by these advances, in 2006 Paul Rothemund generalized the approach into a method called DNA origami, which consists of folding a large ‘scaffold’ strand of DNA with many short ‘staple’ strands in order to generate defined shapes of arbitrary complexity.43 Specifically, designs are created by raster-filling a shape with the scaffold strand and using staple strands that base pair to the scaffold to hold it in place (Fig. 3A).43 In a now-famous publication, Rothemund folded the ~7 kb M13 bacteriophage genome with over 200 32-nt DNA strands into rectangles, stars, and smiley faces (Fig. 3B).43 Rothemund’s technique revolutionized the field by achieving high yields of the designed structures while simultaneously avoiding requirements for the purification of component strands, multiple assembly steps, and exact stoichiometries.43

Follow-on research extended Rothemund’s design principles into 3D with impressive results. Using DNA origami, Ke et al. created DNA cages,44 and Douglas et al. created DNA monoliths, bridges, and crosses.45 Other studies generated 3D structures that twist and curve at the nano-scale. For example, Dietz et al. produced DNA beach balls and square-tooth gears46 (Fig. 3C), and Han et al. produced DNA spheres, shells, and disks (Fig. 3D). A particularly notable example of 3D DNA origami was reported in a publication by Andersen et al., who used the M13 bacteriophage genome as a scaffold to create fully addressable, self-assembling DNA boxes.48 Each box measured 42 × 36 × 36 nm and was folded from six interconnected sheets of DNA with 220 staple strands bridging the edges.48
Furthermore, by programming the lids of these boxes with DNA ‘locks’, the authors showed that they could control their opening with externally supplied DNA ‘keys’ (Fig. 3E).48 In this case, the locks comprised DNA duplexes with sticky ends that facilitated toehold-mediated strand displacement by auxiliary DNA strands.

**Variations on DNA origami**

More recent work has produced variations on the DNA origami technique. For example, Wei et al. developed a 42-nt single-stranded DNA tile that self-assembles into complex 2D patterns, including alphanumeric characters, punctuation marks, and smiley faces, without the need for a scaffold strand (Fig. 3F).49 A recent publication from the same group showed that 32-nt single-stranded DNA tiles, or ‘bricks’, self-assemble into prescribed 3D shapes with up to 10 000 unique components (Fig. 3G).50,51 Another recent study using DNA bricks generated polyhedral assemblies with atomic masses up to 1.2 gigadaltons and long, thick tubes similar in size to some bacilli.52 Because they do not require a scaffold strand, these approaches contrast with Rothemund’s ‘scaffolded’ DNA origami. A separate approach is single-stranded origami, which folds multi-kilobase nucleic...
acid strands into complex 2D patterns without using staple strands. In one example, Han et al. folded hearts from a single strand of DNA approximately 3000 nt in length (Fig. 3H). 53

Another recent example described the combination of single-stranded origami with DNA tiles to fold highly knotted 2- and 3D topologies. 54 Since the advent of DNA origami, semi-automated 55,56 and fully-automated 57,58 approaches for producing target 2- and 3D geometries have emerged. Today, the ease of the origami technique, combined with the commercial availability of chemically synthesized DNA sequences, 59 makes the design and fabrication of complex DNA structures accessible even to non-specialists. 1

Emergence of RNA nanotechnology

In parallel with developments in DNA nanotechnology, the last two decades have witnessed the emergence of the field of ribonucleic acid (RNA) nanotechnology. 60,61 Owing to its 2'-hydroxyl group, RNA is more chemically labile than DNA 62 but it nevertheless has several desirable features for nano-scale fabrication. Like DNA, RNA comprises four nucleotides: adenine, cytosine, guanine, and uracil (A, C, G, and U, respectively). It is highly programmable, with molecular recognition and self-assembly properties governed by canonical Watson–Crick interactions (i.e., C:G and A:U). However, unlike DNA, RNA also engages in many non-canonical interactions (e.g., G:U wobble pairs, sheared G:A pairs, reverse Hoogsteen pairs, and G:A imino pairs), 63,64 which permit the formation of a breathtaking range of complex 3D structures and the execution of catalytic and recognition functions that rival the activities of proteins. 55,66 Indeed, in nature, messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA) have active roles in protein synthesis. 67–70

Additionally, ribozymes, riboswitches, small RNAs, and long, noncoding RNAs are central players in genome replication, intron splicing, regulation of gene expression, epigenetic modification and scaffolding, and more. 66,67

Moreover, RNA structure is organized on the primary, secondary, and tertiary levels. Primary structure is simply the nucleotide sequence of an RNA molecule. Secondary structure comprises recurrent motifs such as helices, hairpins, bulges, internal loops, and multi-way junctions, and tertiary structure comprises noncovalent interactions that connect these motifs together in 3D. 71 Hierarchical folding confers modularity on all levels of RNA structure. 72 Therefore, RNA designers can shop the natural repertoire and mix and match different structural and functional elements ('modules') in order to create composite RNA structures with tailored functionalities. 73 These features, combined with the groundwork laid by research in the field of DNA nanotechnology and advances in chemical RNA synthesis, 74 have enabled the construction of a wide variety of synthetic RNA structures. Important advances in the field of RNA nanotechnology are summarized below.

RNA tectonics

In 1996, Westhof et al. proposed ‘RNA tectonics' to describe the idea that RNA can be resolved into and reassembled from component modules, like a 3D mosaic. 75 A few years later, Jaeger and Leontis put this idea into practice by generating synthetic ‘tectoRNA’ units using a hairpin tetraloop and a tetraloop receptor extracted from the Tetrahymena thermophila group I intron. 76 Their pioneering work showed that the rational placement of interacting loops and loop-receptors could direct the self-assembly of RNA dimers and 1D arrays. 76 Further studies revealed that the self-assembly behavior of tectoRNAs could be fine-tuned by changing the length, helical twist, and flexibility of the linker between interacting motifs. 77 Additionally, by modifying the loop:loop-receptor system with a four-way junction derived from the hairpin ribozyme, Nasalean et al. later demonstrated the self-assembly of long, micrometer-scale RNA structures that resemble actin filaments from the protein world (Fig. 4A). 78

Using a different approach, Horiya et al. showed that kissing loops taken from the genome of human immunodeficiency virus (HIV) could mediate the formation of large RNA assemblies. 79 Kissing loops are short hairpin loops that base pair, and their interactions have been shown to be 102–104 times more stable than loop:loop-receptor interactions. 79 Chworos et al. combined HIV kissing loops with a right angle motif conserved in rRNA to generate RNA squares, square patterns, and finite grids of defined size and shape. 80 In a separate study, a five-way junction derived from class II tRNA was engineered with HIV kissing loops to generate self-assembling, thermostable RNA polyhedra (Fig. 4B). 81 Using 5'-biotinylated tectoRNAs, the authors of this study further illustrated a remarkable degree of spatial control by directing the precise positioning and encapsulation of streptavidins within the RNA structures. 81

Yet other studies have adapted a kissing complex from Escherichia coli (E. coli) with a 120° bend to generate multimeric RNA rings with potential drug delivery applications (Fig. 4C). 82–84 Notably, these circularized RNA assemblies show increased resistance to ribonucleases relative to their linear RNA counterparts. 84 Recent work by Geary et al. has generalized the RNA tectonics approach by composing a ‘syntax' of structural modules, including kissing loops, tail–tail interactions, triple helices, bulges, and three- and five-way junctions. 85 Using this syntax, the authors demonstrated the formation of various RNA shapes, including polygons, ladders, grids, and even hearts 85 that together provide a glimpse into the versatility of RNA as a medium for generating complex nanostructures.

Engineering of viral pRNA

Alongside tectoRNAs, another molecule that has significantly shaped the current RNA nanotechnology landscape is prohead or packaging RNA (pRNA). 60,61 pRNA is a naturally-occurring RNA molecule that derives from the phi29 bacteriophage and related bacteriophages in the phi29-like family. 86 Full-length pRNA is approximately 170 nt long, 87 and it has a conserved secondary structure that features six helical regions, a three-way junction (3WJ), and two kissing loops that mediate pRNA self-assembly in the context of a DNA packaging motor, where pRNA performs an essential but yet unknown function. 87–89 In 1998, Guo et al. demonstrated that the prototype phi29 pRNA

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sequence self-assembles in vitro. More recent studies on phylogenetically related pRNAs have shown that in vitro self-assembly behavior varies by sequence and depends on the nucleotide composition at the 3WJ. Engineering of phi29 pRNA has yielded self-assembling RNA dimers, trimers, and arrays in vitro. The ability to control pRNA self-assembly also has enabled the creation of RNA cages [Fig. 4D] and possible vectors for drug delivery. Recent work has narrowed engineering efforts to the unusually stable 3WJ motif found within the pRNA structure. The phi29 pRNA 3WJ has been used to construct RNA polygons, tetrahedra (Fig. 4E), and prisms. Other studies have developed the 3WJ into a promising platform for the delivery of therapeutic cargoes. To date, only the phi29 pRNA sequence has been developed for RNA nanotechnology. Related or engineered pRNA sequences with different thermodynamic, conformational, and self-assembly properties may prove useful for tailoring the features of pRNA-based structures for specific applications.

**RNA tiles and RNA origami**

As described above, a host of synthetic RNA structures have been engineered by exploiting RNA motifs found in nature. A different approach relies on the de novo design of RNA motifs analogous to the tiles used in DNA nanotechnology. For example, Afonin et al. have demonstrated the self-assembly of RNA PX tiles, and Stewart et al. have shown that RNA DX tiles self-assemble into periodic lattices on the micrometer scale (Fig. 4F). Moreover, Yu et al. have employed RNA T-junctions in the fabrication of self-assembling octameric prisms (Fig. 4G). Another technique that has been developed for RNA nanotechnology is origami. In a recent study, Han et al. demonstrated that single-stranded DNA origami could be adapted for RNA by taking into account the difference in helical periodicity between DNA and RNA molecules. Using the adapted technique, the authors were able to produce structures such as rectangles, rhombuses, and hearts from RNA sequences up to 6.3 kb in length. In another recent study, Qi et al. combined RNA PX tiles with single-stranded origami to fold intricately knotted 2D RNA structures up to 7.5 kb in length.

**Co-transcriptionally folded RNA structures**

RNA folding is fast on the timescale of transcription. Therefore, one exciting prospect for the field of RNA nanotechnology is encoding RNA structures as DNA and subsequently expressing them in vitro or in vivo. In one example, Afonin et al. showed that rationally designed RNA cubes self-assemble from six or 10 component strands during in vitro transcription. In another example, Afonin et al. showed that RNA rings and
Biomedical applications

As described above, numerous advances in nucleic acid nanotechnology have contributed to the development of supramolecular DNA and RNA assemblies with precise structural and dynamic control. Here, we highlight examples of high-order nucleic acid structures in two promising biomedical applications: biosensing and therapeutic drug delivery.

Biosensing

Biosensors are tools that convert signals from biological analytes (e.g., cells, proteins, nucleic acids, and small metabolites) into recordable signals. They consist of a recognition component (i.e., a probe), a transducer, and a signal amplification device. One challenge in biosensing is facilitating maximum interactions between analytes and probes while preventing agglomeration due to an irregular distribution of probes across a sensing surface. Rationally designed DNA tetrahedra have proven particularly useful for addressing this problem. DNA tetrahedra are mechanically robust structures that self-assemble rapidly and in nearly quantitative yields from four component strands. In 2010, Pei et al. developed a sensitive electrochemical biosensor using DNA tetrahedra assembled from three DNA strands bearing a terminal thiol group (−SH) and one DNA strand containing a probe. The tetrahedra self-assembled on a gold (Au) electrode by Au–S chemistry and were shown to enforce uniform probe-to-probe spacing. Additionally, tetrahedra with pendant DNA probes showed a lower detection limit for target DNA of approximately 1 picomolar (pM), which represented a 250-fold improvement over the same probe without tetrahedron scaffolding. Moreover, aptamers are synthetic nucleic acid sequences that are capable of organizing proteins in a hydrogen-producing biosynthetic pathway. Other studies have extended these principles into living systems. For example, Delebecque et al. co-transcriptionally assembled RNA scaffolds in E. coli that were capable of organizing proteins in a hydrogen-producing biosynthetic pathway. More recently, Li et al. showed that an RNA double square design could be cloned, expressed, and folded in E. coli (Fig. 4H). Compared to conventional origami techniques, the gene expression of RNA assemblies avoids costly chemical synthesis as well as lengthy annealing steps and moves the field a step closer to one of the holy grails of RNA nanotechnology, i.e., rationally designing RNA objects as large and complex as natural RNA machines.

Drug delivery

Owing to their intrinsic biocompatibility, nucleic acids also hold promise as platforms for therapeutic drug delivery. In 2006, Erben et al. illustrated the potential of DNA structures to serve as molecular containers for therapeutic cargo by loading cytochrome c into a self-assembled DNA tetrahedron. Follow-on research showed that DNA tetrahedra are stable to enzymatic degradation, and DNA tetrahedra, icosahedra, and cages may be reconfigured in response to external signals for controlled drug release. Furthermore, many studies have demonstrated that DNA structures are taken up by cultured cells without transfection reagents. Recently, Wiraja et al. tested a variety of nano-scale DNA structures, including tetrahedra, cylindrical rods, rectangles, and triangles, and showed that highly-ordered structures smaller than 75 nm penetrate the skin. Using a mouse model of melanoma, the study further showed that DNA tetrahedra loaded with the chemotherapeutic drug doxorubicin could achieve over 2-fold higher drug accumulation and tumor inhibition relative to topically applied doxorubicin and liposome- or nanoparticle-formulated doxorubicin.

While some DNA structures achieve passive uptake, other platforms have been developed with active targeting mechanisms to promote uptake by specific populations of cells. In one example, Chang et al. showed that DNA icosahedra self-assembled from five- and six-point star motifs could be functionalized with a MUC 1 aptamer for targeted delivery of doxorubicin to MUC 1+ MCF-7 breast cancer cells. In a separate, pioneering study, Lee et al. developed a DNA tetrahedron with a small interfering RNA (siRNA) hybridized to each edge (Fig. 5B). SiRNAs are short, double-stranded RNA molecules that elicit potent gene silencing by co-opting an endogenous RNA interference (RNAi) pathway. Currently, major challenges for the delivery of oligonucleotide drugs such as siRNAs include nuclelease protection, systemic delivery, and targeted cellular uptake. Notably, siRNA-functionalized tetrahedra administered in a mouse tumor xenograft model...
displayed longer half-lives in blood circulation relative to siRNAs alone. Additionally, tetrahedra with siRNAs bearing folate distributed to several tissues but accumulated in folate receptor-overexpressing KB cells, where they efficiently silenced a luciferase reporter. In line with these results, other studies have shown that DNA structures can confer stability on their therapeutic payloads while also maintaining or even improving their therapeutic efficacies.

Yet other DNA platforms have been developed with sophisticated mechanisms for cargo release. In a recent example, Bujold et al. designed DNA ‘nanosuitcases’ that encapsulate siRNA and release it in the presence of specific mRNA or miRNA triggers (Fig. 5C). In another example, Douglas et al. developed DNA origami ‘nanorobots’ with AND logic gates. These logic gates consisted of two aptamer ‘locks’ that triggered a drastic reconfiguration of the robot and exposed molecular payloads upon binding both antigenic ‘keys’. Recent work by Li et al. combined both active targeting and controlled release mechanisms in a DNA nanorobot loaded with thrombin and functionalized with nucleolin-binding aptamers (Fig. 5D). Thrombin is a protease that induces coagulation and may be useful for starving tumors of nutrients and oxygen by selective occlusion of tumor blood vessels. Nucleolin is a protein that is expressed on tumor-associated endothelial cells. Remarkably, the nanorobots were capable of depositing thrombin at tumor cells in mouse models of breast cancer, and they also proved safe and immunologically inert in both mice and miniature pigs.

Several high-order RNA structures also have been developed for therapeutic drug delivery. Because RNA is more chemically labile than DNA, RNA-based platforms often are chemically modified to improve their stability under physiological conditions. Common modifications include substitutions at the 2’ position of ribose as well as in the internucleotide linkage. A series of studies has developed RNA cubes designed in silico as a promising platform for drug delivery.
The cubes are precisely controlled in terms of size, shape, and composition; they can be chemically modified for downstream applications; and they also are capable of self-assembly in isothermal conditions during in vitro transcription or from several short strands following chemical RNA synthesis.\textsuperscript{118,119} Computational and experimental analyses have shown that cube designs with 10 bp per edge and single-stranded regions in the corners are not strained and assemble efficiently.\textsuperscript{166} In 2011, Afonin et al. developed a protocol for the design and self-assembly of siRNA-functionalized RNA cubes using processes that are fully automatizable.\textsuperscript{167} The cubes interact with recombinant human Dicer in vitro to produce siRNAs\textsuperscript{167} (Fig. 5E). Furthermore, a recent study by Afonin et al. showed that siRNA-functionalized RNA cubes are capable of triggering RNAi in GFP-expressing MDA-MB-231 breast cancer cells and reducing HIV-1 production in HeLa cells.\textsuperscript{168}

Finally, another promising platform for drug delivery has come from research on pRNA and its thermodynamically stable 3WJ.\textsuperscript{60,61} The phi29 pRNA 3WJ has been engineered to deliver a variety of therapeutic agents, including small molecules,\textsuperscript{108} miRNAs,\textsuperscript{109} anti-miRNAs,\textsuperscript{110,111} and siRNAs.\textsuperscript{99,112,113} In a recent example, Xu et al. combined the phi29 pRNA 3WJ with a four-way junction derived from the hairpin ribozyme to generate 2′-deoxyfluoro U/C modified RNA pyramids (Fig. 5F).\textsuperscript{169} These pyramids were functionalized with the chemotherapeutic drug paclitaxel via photocleavable spacers, and irradiation with ultraviolet light induced drug release and cytotoxicity in MDA-MB-231 breast cancer cells.\textsuperscript{169} In a different example, Li et al. reported the self-assembly of a nuclease-resistant, 2′-deoxyfluoro U/C modified RNA tetrahedron based on the phi29 pRNA 3WJ (Fig. 5G).\textsuperscript{106} Functionalization of the tetrahedron with ribozymes, aptamers, and siRNAs did not disrupt its structure, and the functional modules retained the capacity for ribozymatic cleavage, ligand binding, and gene knockdown in vitro.\textsuperscript{106} Furthermore, this study showed that tetrahedra functionalized with an aptamer against epidermal growth factor receptor (EGFR) and an siRNA were capable of internalizing into tumor tissue and silencing a luciferase reporter in a mouse model of breast cancer.\textsuperscript{106}

Outlook

From the development of DNA junctions, tiles, and origami in DNA nanotechnology to the development of tectoRNAs, engineered pRNA, and co-transcriptionally folded structures in RNA nanotechnology, research over the past 40 years has expanded the possibilities for creating supramolecular nucleic acid architectures with precise structural and dynamic control. Today, high-order DNA and RNA structures are taking their place as powerful tools with promising biomedical applications. In biosensing, nano-structured DNA platforms boost sensitivity for the detection of biological analytes while preventing agglomeration. In drug delivery, DNA- and RNA-based platforms offer potential solutions to formidable in vivo challenges, including nuclease protection, systemic delivery, and targeted cellular uptake for oligonucleotide drugs. Many of the achievements in nucleic acid nanotechnology would not be possible without parallel achievements in chemical DNA and RNA synthesis, which have given ready access to the oligonucleotide sequences necessary for constructing nucleic acid assemblies.\textsuperscript{1} Still, for bottom-up fabrication using RNA, sequence length and yield are considerable limitations. RNA assemblies made by gene expression may circumvent these issues and facilitate applications in vivo. Additionally, the cost of oligonucleotide synthesis is decreasing. In fact, a relative of Moore’s law has been reported for the effective cost of DNA, which is halved every 30 months.\textsuperscript{170} Continued developments in oligonucleotide synthesis, including in the production of longer sequences for lower costs, will be important for supporting efforts in DNA and RNA nanotechnology and for realizing the extraordinary potential of high-order nucleic acid structures for biomedical applications and beyond.

Conflicts of interest

A. C. H. is a co-inventor and applicant on a patent for viral pRNA 3WJ sequences.

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