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The emerging field of RNA nanotechnology

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Like DNA, RNA can be designed and manipulated to produce a variety of different nanostructures. Moreover, RNA has a flexible structure and possesses catalytic functions that are similar to proteins. Although RNA nanotechnology resembles DNA nanotechnology in many ways, the base-pairing rules for constructing nanoparticles are different. The large variety of loops and motifs found in RNA allows it to fold into numerous complicated structures, and this diversity provides a platform for identifying viable building blocks for various applications. The thermal stability of RNA also allows the production of multivalent nanostructures with defined stoichiometry. Here we review techniques for constructing RNA nanoparticles from different building blocks, we describe the distinct attributes of RNA inside the body, and discuss potential applications of RNA nanostructures in medicine. We also offer some perspectives on the yield and cost of RNA production.

Macromolecules of DNA, RNA and proteins have intrinsically defined features on the nanoscale and may serve as powerful building blocks for the bottom-up fabrication of nanostructures and nanodevices. The field of DNA nanotechnology[1–3] is now well established, having its origins in work by Seeman some 30 years ago, and peptides and proteins have also been studied for applications in nanotechnology[4–7]. The concept of RNA nanotechnology[8–15] has been around for more than a decade, and the first evidence for the construction of RNA nanoparticles through the self-assembly of several re-engineered natural RNA molecules was reported in 1998[8]. However, interest in RNA nanotechnology has increased in recent years as recognition of its potential for applications in nanomedicine — including the treatment of cancer, viral infection and genetic diseases — has grown (Fig. 1).

RNA can be designed and manipulated with a level of simplicity that is characteristic of DNA, while displaying flexibility in structure and diversity in function (including enzymatic activities) that is similar to that of proteins. Although RNA nanotechnology is similar to that of DNA in a number of ways, there are important differences between the two disciplines (Table 1).

RNA is a polymer made up of four different nucleotides: adenine (A), cytosine (C), guanine (G) and uracil (U), whereas DNA contains thymine (T) rather than U. And as well as the Watson–Crick base pairing found in DNA (A with T, C with G), other forms of base pairing (referred to as non-canonical base pairing) are possible, such as G with A or U, which allows RNA to fold into rigid structural motifs that are distinct from those formed by single-stranded DNA[10,12,16–24] (Fig. 2). At present, an RNA strand containing up to 80 nucleotides can be synthesized commercially, and an 80-nucleotide RNA strand can display up to 4^{80} (or 10^{48}) different structures. The availability of so many different structural building blocks is an advantage for many applications.

Moreover, RNA typically contains a large variety of single-stranded stem-loops for intra- and/or intermolecular interactions, and these can be used to make 'dovetail' joints between different building blocks, thus removing the need for an equivalent to dowels in RNA nanostructures and nanomachines. Loops and motifs also allow for the construction of a more complicated secondary structure. Furthermore, RNA molecules such as aptamers, ribozymes and short interfering RNA (siRNA) can have special functionalities (see 'Applications of RNA nanotechnology' section).

Among the three helices (RNA/RNA, RNA/DNA and DNA/DNA), the RNA/RNA double helix is the most stable[25,26]. RNA motifs and modules with special bends or stacks are particularly stable. The thermodynamic stability has been defined as the free energy, G, required for complex formation, or in some cases, to unwind the helix ($\Delta G^0 = -G^0_{helix} = G^0_{unwind}$); thus, the lower the free energy ($-G^0_{helix}$) the complex holds, the more stable it is. Because ΔG^0 is affected by neighbouring sequences, $-G^0_{helix}$ for RNA is calculated to be lower than DNA[25,26] based on the nearest-neighbour model (Table 1). However, under physiological conditions, the RNA helix displays A-type configuration whereas the DNA helix is predominantly B-type. The 2′-OH in RNA ribose locks the ribose into a 3′-endo chair conformation that does not favour a B-helix. Base stacking is governed by van der Waals interaction, which contributes directly to the enthalpy. Though the difference in the stacking interaction is small between DNA and RNA, the sum over numerous base pairs can make a difference to the helix stability. Thus, RNA nanoparticles are more stable thermodynamically than their DNA counterparts. Like DNA tiles, stable RNA helices in solution can be produced using four to six nucleotides of RNA[12], but in certain cases as few as two nucleotides can promote complex formations in RNA[27–31].

Distinct attributes of RNA inside the body

Therapeutic particles are initially recognized by cell-surface receptor(s) before being internalized through the plasma membrane into vesicles (called endosomes) that sort the particles for either degradation or recycling. Escaping the endosome is an important consideration for *in vivo* delivery because most molecules cannot survive its acidic environment, with a pH ranging from 4.3 to 5.8 (ref. 32). At this pH, protonation of DNA purine bases leads to their removal (a process known as depurination) and the resulting apurinic DNA is susceptible to cleavage[33]. The higher stability of RNA at low pHs is especially useful in therapy because it means that they will survive in the endosome and disperse throughout the cell after entry (Table 1).

Another intriguing property of RNA is the possibility of producing self-assembled RNA nanoparticles *in vivo*. In contrast to DNA, small RNA molecules are transcribed in the cell using DNA as a template. By using an inducible promoter[34] and appropriate terminators for transcription, small RNA molecules can be produced controllably. RNA can be processed into the desired length by including delta ribozymes at both the upstream and downstream terminals

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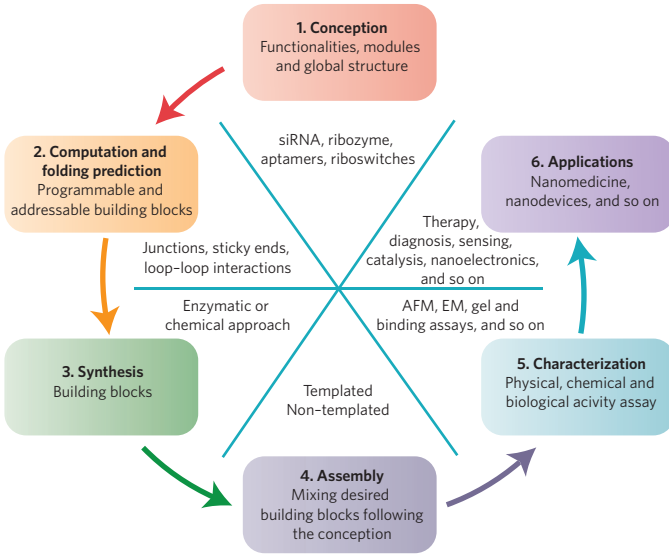


Figure 1 | Approaches to RNA nanotechnology. The construction of RNA nanoparticles is a multistep process that starts with a conception step in which the desired properties of the nanoparticle are defined. A computational approach is then applied to predict the structure and folding of the building blocks and the consequences of inter-RNA interactions in the assembly of RNA nanoparticles. After the monomeric building blocks are synthesized (either by enzymatic or chemical approaches), the individual subunits assemble into quaternary architectures by either templated or non-templated methods. The assembled RNA nanostructures are characterized (by atomic force microscope (AFM), electron microscope (EM), gel electrophoresis and so on) to ensure proper folding with desired structural and functional capabilities. After thorough evaluation, the nanoparticles will be used for various applications.

for *cis*-cleavage[35]. Natural RNA nanoparticles such as dimers[36–39] and hexamers[8,9] have been discovered in cells. Sequences such as packaging RNA (pRNA)[35] or transfer RNA (tRNA)[40,41] for guiding the self-assembly of RNA nanoparticles with functionalities such as siRNA[42,43], ribozymes[35], or aptamers[40] can be incorporated in the DNA template *in vivo*[35].

Small RNAs, such as riboswitch, with regulatory functions[44–47] in the cell may be viewed as Boolean networks based on logic operations[48,49]. Input nodes can be seen as RNA nanostructures and the output (for example, the activation of a pathway) is based on logic functions of input RNA concentrations. Numerous small RNA regulators can be used to regulate the *in vivo* products and functional pathways, with controls by induction or repression through the *trans*- and *cis*-actions. Varieties of small RNA can work cooperatively, synergistically, or antagonistically — based on the design — to produce computational logic circuits as conjunctive or disjunctive normal forms, or other kinds of logic operation. By designing the logic network of AND/NOT/OR gates in the cell, an 'RNA computer' can theoretically be implemented and applied to bacterial, yeast and mammalian systems[48,49].

Techniques for constructing RNA nanoparticles

Construction of nanoparticles requires the use of programmable, addressable and predictable building blocks. Self-assembly of RNA building blocks in a predefined manner to form larger two-, three- and four-dimensional (2-, 3-, 4D) structures is a prominent bottom-up approach and represents an important means by which biological techniques and biomacromolecules can be successfully integrated into nanotechnology[12,50,51].

Within the realm of self-assembly there are two main subcategories: templated and non-templated assembly. Templated assembly involves the interaction of RNAs with one another under the influence of a specific external force, structure, or spatial constraint. RNA transcription, hybridization, replication, moulding and phi29 pRNA hexameric-ring formation are all in this category. Non-templated assembly involves the formation of a larger structure by individual components without any external influence. Examples include ligation, chemical conjugation, covalent linkages, loop–loop interactions of RNA such as the HIV kissing loop and the phi29 pRNA dimer or trimer formation[10,12,50–52]. Various approaches available for RNA nanoparticle construction are discussed below (see Fig. 3 for summary).

The first tactic uses the assembly mechanism of natural RNA nanoparticles that can form specific multimers *in vivo*. For example, the retrovirus kissing loops facilitate genomic RNA dimerization[36,37]. The pRNA of the bacteriophage phi29 DNA-packaging motor assembles into dimers and hexamers through hand-in-hand interactions between two right and left interlocking loops[8,12,28,39,52,53]. The *bcd* mRNA of *Drosophila* embryos forms dimers through hand-in-arm interactions[38]. *E. coli* non-coding RNA *dsrA* assembles into stripe patterns through their built-in palindrome sequence[23]. The assemblies of RNA nanoparticles *in vitro* that mimic their natural counterparts were reported twelve years ago[8]. The unusual HIV kissing-loop mechanism has also inspired the design of tectoRNA architectures[13,29].

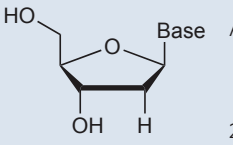
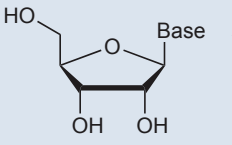
The second tactic is to import some of the well-developed principles from DNA nanotechnology. Although RNA is different from DNA, there are some common structural and chemical features that can be exploited for progressing RNA nanotechnology.

DNA nanotechnology uses the nature of DNA complementarity for the construction of nanomaterials by means of intermolecular interactions of DNA strands. A variety of elegant shapes have been created with precise control over their geometries, periodicities and topologies[1–3] (Fig. 2). Various crossover motifs have been designed through reciprocal exchange of DNA backbones[3]. Branched DNA tiles have been constructed using sticky ends and crossover junction motifs, such as tensegrity triangles (rigid structures in periodic-array form)[54] and algorithmic self-assembled Sierpinski triangles (aperiodic arrays of fractal patterns)[55]. The DNA tiles can further self-assemble into nanotubes, helix bundles[56] and complex DNA motifs and arrays for positioning nanoparticles, proteins or dyes with precise control, such as polycatenated DNA ladders[57]. Elegant 3D DNA networks using a minimal set of DNA strands with topologies such as cubes, polyhedrons, prisms and buckyballs have also been fabricated based on junction flexibility and edge rigidity[3,58]. Continuous growth of the tensegrity triangle in the periodic DNA module has resulted in the formation of DNA crystals diffracting to 4-Å resolution[59].

A striking illustration of the addressable and programmable properties of DNA is Rothemund's DNA origami[60], where a long single-stranded viral DNA is used as a scaffold for binding shorter strands to generate well-defined 2D and 3D configurations. DNA origami was subsequently applied to build 3D boxes that can be locked and unlocked[61], nano-arrays for label-free detection of substrates[62] and to elucidate the structure of organized proteins[63]. Rationally designed supramolecular DNA assemblies can be conjugated with organic and inorganic molecules, such as conjugation of porphyrins on parallel DNA helix bundles[64], nanomagnets[65] and elegant nanomachines[58,66]. Replicable DNA architectures have been achieved to scale-up the production of DNA nanostructures for practical applications by using enzymatic rolling-circle replication, bacterial cells infected with a viral vector[67], or chemical approaches for amplifying branched DNA arms[68].

Although the folding properties of RNA and DNA are not exactly the same, the fundamental principles in DNA nanotechnology are applicable to RNA nanotechnology. For example, the use of the three-way junction (3WJ) and four-way junction (4WJ)[18,29] to build new and diverse RNA architectures is very similar to the branching

Table 1 | Differences between DNA and RNA.

	DNA	RNA
Elements	 A, C, G, T; 2′-deoxyribose	 A, C, G, U; ribose
Base pairing	Canonical Watson–Crick (W–C)	Canonical and non-canonical W–C
Acidic effect	Depurination: apurine DNA sensitive to cleavage	Stable
Alkaline effect	Stable up to pH 12	Sensitive to alkaline hydrolysis
Configuration	Predominantly B-form: -base pairs/turn of the helix: 10.5; -pitch: 3.5 nm; -helix rise/base pairs: 0.314 nm; -humidity: nucleotide: H_2O = 1:1	A-form: -base pairs/turn of the helix: 10.9; -pitch: 2.5 nm; -helix rise/base pairs: 0.275 nm; -humidity: nucleotide: H_2O = 1:0.7
Chemical stability	Relatively stable but sensitive to DNase	Unstable, sensitive to RNase, but stable after chemical modification, for example, 2′-F or 2′-OMe modification
Thermal stability	G:C more stable than A:T	Thermally more stable than DNA, especially for RNA motifs and modules with particular bends or stacks
Free energy, ΔG^0	−1.4 KJmol^{-1} per base pair stack[25]	−3.6 to −8.5 KJmol^{-1} per base pair stack[25]
Helix formation	Needs a minimum of four nucleotides	Needs a minimum of two nucleotides[26,27]
Intermolecular interactions	Cohesive ends, crossover motifs	Cohesive ends, crossover motifs, kissing loops, interlocking loops
In vivo replication		
Initiation	Origin of replication with primer	Promoter, exact nucleotide to start without primer
Termination	No nature sequence for replication termination	Specific transcription terminators
In vitro synthesis		
Enzymatic	DNA polymerase, polymerase chain reaction (PCR)	T7/SP6 transcription
Chemical	Up to 160 nucleotides; low cost	Up to 117 nucleotides; high cost and low yield

approaches in DNA[1,3] (Fig. 2a,b,e,f). Both RNA and DNA can form jigsaw puzzles[13,69] and be developed into bundles[12,23,30,70] by combining elongation and expansion in the *x*–*y* direction (Fig. 2a,b,g–j). The finding that insertion of bulges in the RNA helix leads to the formation of twisted bundles[12] (Fig. 2i) was later demonstrated in DNA (Fig. 2g), revealing that insertions and deletions of bases can form twisted DNA bundles with handedness[70], thereby illustrating the same basic principle. However, RNA is more rigid in bulge structure owing to non-canonical interactions, whereas in DNA the twisting requires the interaction of two DNA helices with four strands[70].

Recently, RNA cubic scaffolds[71] were constructed using several RNA sequences that do not fold on themselves but self-assemble with one another in a defined manner. This strategy is reminiscent of DNA nanotechnology, but in contrast to DNA strategies, RNA synthesis can be coupled to RNA self-assembly to generate fully assembled RNA cubes during *in vitro* transcription.

The third tactic is to apply computational methods in the construction of RNA nanoparticles. Computational approaches can be used to guide the design of new RNA assemblies and to optimize sequence requirements for the production of nanoscale fabrics with controlled direction and geometry[37,72–75]. In contrast to traditional methods in which raw materials are selected rather than designed for a given application, the next generation of building blocks can be designed *a priori* for programmed assembly and synthesis. There are two steps involved in building RNA nanoparticles. The first is a computational approach (for example, using Kinefold[72]) using the spontaneous self-folding property of RNA into defined structures through base–base interactions dependent on their characteristic ΔG (ref. 76). The second is the spontaneous assembly of the resulting RNA building blocks into larger assemblies based on the predicted architecture. This creates an effective computational pipeline for generating molecular models of RNA nanostructures. A recent example is the construction of cubic RNA-based scaffolds, whereby RNA sequence designs were optimized to avoid kinetic traps[77].

The fourth tactic is to use the existing RNA structure with known function as building blocks in RNA-nanoparticle construction. Varieties of mechanisms in RNA loop–loop interactions[8,12,31], tertiary architecture contacts[12,15,30] and formation of special motifs[12,21,29–31,78–82] have been elucidated. Building blocks are first synthesized after computing intra- and intermolecular folding. Nanoparticles are built through spontaneous templated or non-templated self-assembly as planned. A rich resource of well-developed databases can be used to extract known RNA structural units for the construction of new RNA nanoparticles with desired properties[37,83,84].

Several methods have borrowed the properties of RNA in loop–loop interactions to construct RNA nanoparticles. The first method is based on the structural features of the pRNA of the bacteriophage phi29 DNA-packaging motor[8,85], which uses a hexameric RNA ring to gear the machine[28,86,87]. The pRNA has been re-engineered to form dimers, trimers, tetramers, hexamers and arrays through hand-in-hand or foot-to-foot interactions between two interlocking loops[12,52] (Fig. 4). Dimers are formed using two building blocks with Ab′ (right and left hand, respectively) and Ba′ (Fig. 4b). Trimers are formed using three building blocks with Ab′, Bc′ and Ca′[12,50,51] (Fig. 4c). Dimers of an extended configuration (twins) can also be efficiently self-assembled by introducing a palindrome sequence into the 3′-end of the pRNA[12]. These nanoparticles have been used successfully as polyvalent vehicles

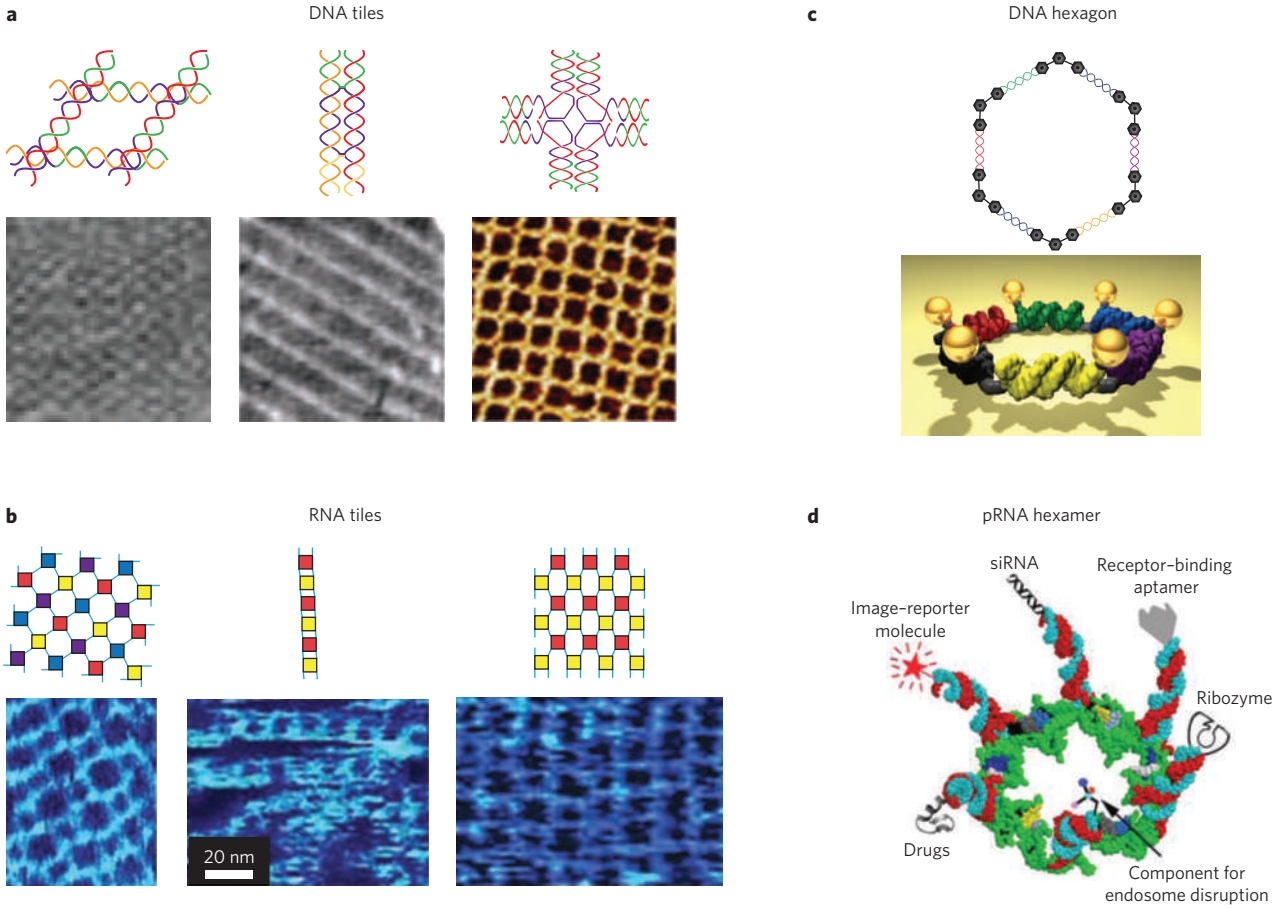


Figure 2 | Comparison of self-assembled DNA and RNA nanoparticles. a,**b**, Representative transmission electron microscope (TEM) and AFM images of DNA and RNA tiles are shown below the corresponding cartoon models. **a**, Left to right: TEM image of a parallelogram DNA tile formed by joining four Holliday junctions in parallel; TEM image of a double helix tile formed by the exchange of two DNA duplex strands and AFM image of a cross-shaped tile[1,2] possessing four arms. **b**, Left to right: AFM images of tectosquare nanopatterns[13] (striped velvet, ladder and fishnet pattern). **c**, Illustration of a hexagonal array of gold nanoparticles (yellow circles in lower image) on a DNA hexagon consisting of six non-identical molecules (triplets of grey hexagons in upper image)[1,2], each linked with two single-stranded DNA molecules (coloured lines). **d**, Illustration of a pRNA hexameric ring containing six positions that can carry different molecules[50,87]. **e**,**f**, Illustration of various 3D DNA polygons (**e**)[2] and RNA cubic scaffolds (**f**)[30,77]. **g**, TEM images of DNA bundles[70]. **h**,**i**, AFM images of RNA bundles (**h**)[23] and pRNA arrays (**i**)[12]. **j**, 3D model of H-shaped tectoRNA[21]. Figures reproduced with permission from: **a**, ref. 1, © 2009 ACS and ref. 2, © 2008 AAAS; **b**, ref. 13, © 2004 AAAS; **c**, ref. 1, © 2009 ACS and ref. 2, © 2008 AAAS; **d**, ref. 50, © 2005 Mary Ann Liebert; **e**, ref. 2, © 2008 AAAS; **f**, refs 30 and 77, © 2010 NPG; **g**, ref. 70, © 2009 AAAS; **h**, ref. 23, © 2009 ACS; **i**, ref. 12, © 2004 ACS; **j**, ref. 21 © 2006 Oxford Univ. Press.

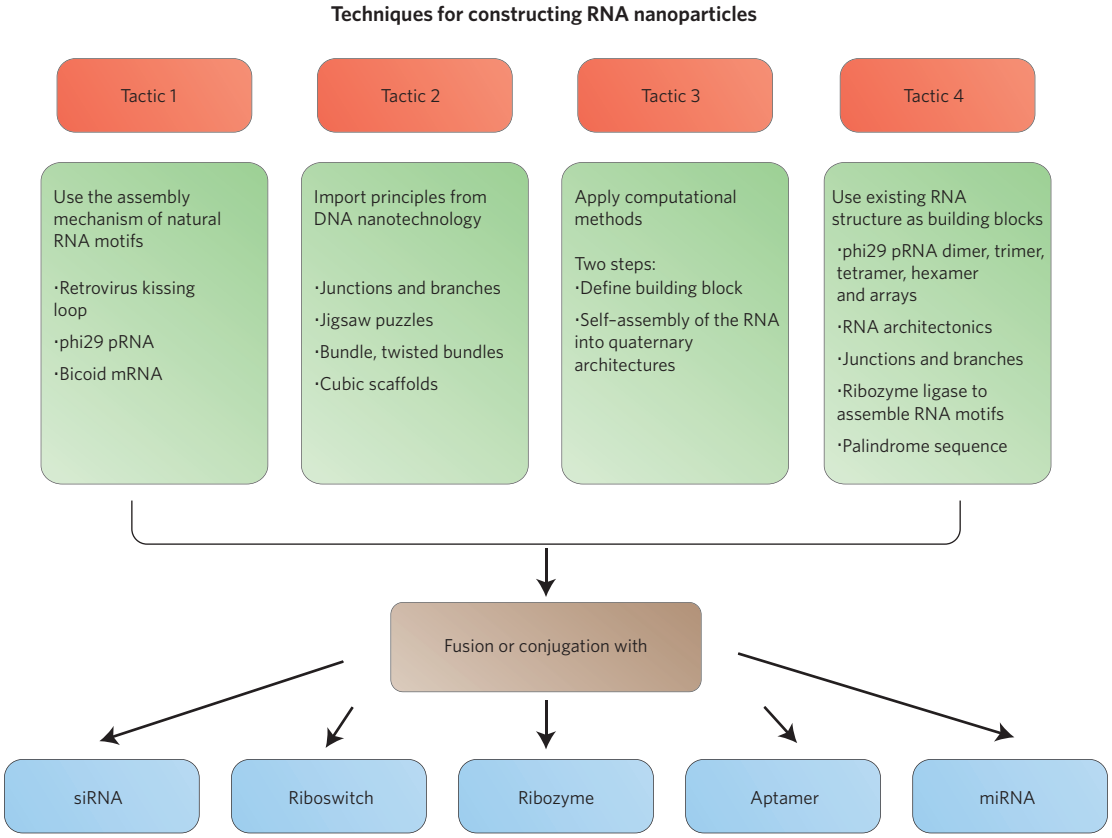


Figure 3 | Summary of different techniques for constructing RNA nanoparticles. See 'Techniques for constructing RNA nanoparticles' for complete description.

to deliver a variety of therapeutic molecules (see 'Applications of RNA nanotechnology' and 'Challenges and perspectives' sections)[12,52]. The use of pRNA as building blocks for the construction of RNA arrays has also been achieved[12]. When three twins, Ab′, Bc′ and Ca′ are mixed, loop–loop interlocking makes the particles grow in three dimensions.

The second method is the RNA 'architectonics'[13], whereby structural modules specifying bends or stacks can be encoded in artificial RNA sequences for self-assembling higher-order specific shapes of RNA. Examples include RNA filaments[10,21,24] (Fig. 2j), molecular jigsaw-puzzle units called tectosquares[13,29] (Fig. 2f) and tRNA antiprisms[88].

The third method is the application of 3- and 4WJs that are selected from known RNA structures or motifs[18,19] to serve as the cornerstone in nanoparticle construction (Fig. 2)[29,76]. Some examples include: RNA-structural motif (from ribosomal RNA; rRNA) to guide the tetramer assembly of L-shaped tectoRNAs; 3WJ motifs (from 23S rRNA) to construct a T-shaped arrangement of three helices; and tRNA motifs consisting of 4- and 5WJs to fold L-shaped tertiary structures[29,37].

The fourth method is to assemble non-natural functional RNAs with defined 3D structures using synthetic ribozyme ligase by employing the molecular design of RNA based on the *in vitro* selection technique[16,17]. Conformational switch of RNA nanostructures can also be constructed using a peptide-binding RNA structural motif[20].

The fifth method is the use of a palindrome sequence that differs from the sticky end, at the 5′- or 3′-end of the RNA. The molecule will spontaneously assemble through self-annealing of the palindrome sequence immediately after *in vitro* transcription or chemical synthesis, before purification[12]. This method is useful for the creation of bundles, especially for designing 3D branches. As each of the 11 nucleotides of the A-RNA generates one helical turn of 360°, the angle or the direction of RNA-fibre extension is controllable by varying the number of nucleotides in the helix containing the palindrome sequence.

Applications of RNA nanotechnology

The versatility of RNA structure, low free energy in RNA annealing, amenability in sequence, options for structure control, and the property of self-assembly make RNA an ideal material for nanotechnology applications. It is possible to adapt RNA to construct ordered, patterned, or preprogrammed arrays or superstructures (Fig. 2h,i). RNA sequences can mediate the growth of hexagonal palladium nanoparticles[89]; programmable self-assembling properties of RNA ladders can direct the arrangement of cationic gold nanoparticles; and periodically spaced RNA architectures can serve as a scaffold for nanocrowns[90]. Geometrically symmetrical shapes such as dimers, trimers or polygons can be constructed from RNA[12,13,52] (Fig. 4). As symmetrical shapes facilitate the formation of crystals, RNA might serve as scaffolds for X-ray crystallography. Furthermore, self-assembly interaction between interlocking loops, self-linkages through a palindrome sequence, the continued growth into a hierarchical structure, and ease in conjugation and biocompatibility make RNA a good candidate for the construction of scaffolds for tissue engineering[12,21,23]. Several laboratories have developed RNA aptamers as biosensors[91].

RNA's new role in nanomedicine applications include cell recognition and binding for diagnosis[92]; targeted delivery through receptor-mediated endocytosis[93]; and intracellular control and computation through gene silencing and regulation[48,49], nuclear membrane penetration, and blood–brain barrier passing[94]. The most important therapeutic RNA moieties are discussed below.

An siRNA[42,43] helix has 20–25 nucleotides and it interferes with gene expression through the cleavage of mRNA by a protein–RNA

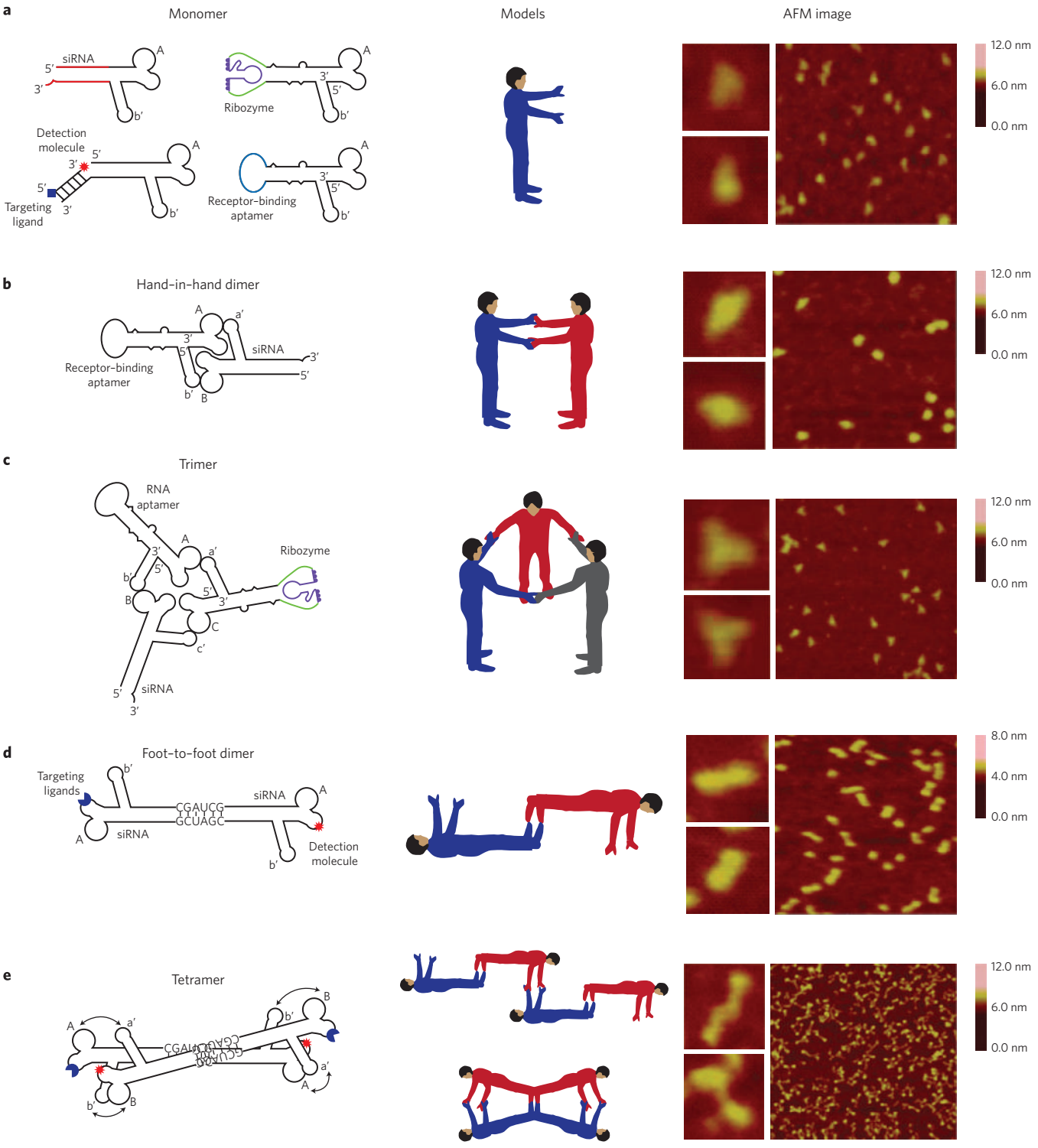


Figure 4 | Applications of RNA nanotechnology. Left to right column: Schematic, models and AFM image showing the formation of different therapeutic nanoparticles containing siRNA, ribozymes, aptamers and other moieties using bacteriophage phi29 pRNA that possess left- and right-hand interlocking loops or a palindrome sequence[8,12,39]. **a**, pRNA monomers bearing either a ribozyme, a receptor-binding aptamer, or a targeting ligand and detection molecule. Uppercase and lowercase letters represent right and left hand, respectively. Same letter pair (for example, Aa′) indicates complementarity[28]. **b**, Monomer Ab′ that contains a receptor-binding aptamer and monomer Ba′, which contains an siRNA, assemble to form hand-in-hand dimers. **c**, Trimers are formed between monomer Ab′ (which contains an RNA aptamer), Bc′ (contains an siRNA) and Ca′ (contains a ribozyme). **d**, Foot-to-foot dimers form through the palindrome sequence at the end of two Ab′ monomers, with one bearing a targeting ligand and the other a detection molecule. **e**, Tetramers assemble by the combination of interlocking loops and palindrome mechanism of two dimers (Ab′ and Ba′). The models illustrate how the various structures are held together. Frame size for AFM images: 200 × 200 nm (**a**, **b**); 300 × 300 nm (**c**); 250 × 250 nm (**d**); 500 × 500 nm (**e**). Figures reproduced with permission from: **a**–**e**, ref. 12, © 2004 ACS and ref. 52, © 2003 ASP.

complex called RNA-induced silencing complex (RISC). The siRNA specifically suppresses the expression of a target protein whose mRNA includes a sequence identical to the sense strand of the siRNA. This discovery led to the award of the 2006 Nobel Prize to Andrew Fire and Craig Mello[42].

A ribozyme[95,96] is an RNA molecule that has enzymatic activity. Ribozymes have significant therapeutic potentials capable of regulating gene function by intercepting and cleaving RNA substrates, such as mRNA, or the viral genome of RNA containing a sequence complementary to the catalytic centre of the ribozyme. This discovery also led to the award of the 1989 Nobel Prize to Thomas Cech and Sydney Altman.

RNA aptamers[97,98] are a family of oligonucleotides with functions similar to that of antibodies in their ability to recognize specific ligands (organic compounds, nucleotides, or peptides) through the formation of binding pockets[92]. Systematic evolution of ligands by exponential enrichment (SELEX)[99] is the method used to screen for the aptamers from randomized RNA pools developed *in vitro* by Ellington and Szostak[97] and by Tuerk and Gold[98]. Using this technique, various aptamers have been selected for targeting markers relevant to diseases[92,100,101].

Riboswitches[102] are RNA components that bind small molecules and control gene expression in response to an organism's needs. As a biological control mechanism, riboswitches can recognize metabolites, induce premature termination of mRNA transcription, block ribosomes from translating mRNAs, cleave mRNAs and even trigger mRNA destruction. Therefore, RNA switches can be re-engineered to create a new generation of controllers regulated by drug-like molecules to tune the expression levels of targeted genes *in vivo*. Such RNA-based gene-control machines hold promise in future gene therapies by supplying nanoscale *cis*-acting modulation[103,104].

Various RNA moieties including siRNAs, ribozymes, antisense RNAs, aptamers and riboswitches, as well as other catalytic or editing RNAs, can be easily fused or conjugated into RNA nanoparticles (Fig. 4). The advantages of RNA nanomedicine include: (1) self-assembly (see 'Techniques for constructing RNA nanoparticles' section for self-assembly and self-processing *in vivo*); (2) multivalency; (3) targeted delivery; (4) protein-free; (5) nanoscale size; (6) controlled synthesis with defined structure and stoichiometry; and (7) combining therapy and detection of therapy effects into one particle.

Bottom-up assembly of RNA can lead to multivalency[51]. Each subunit may be separately functionalized to carry different therapeutic payloads, reporters and/or targeting ligands (Fig. 2d; Fig. 4a). Cell-type-specific delivery allows a lower concentration of the drug to be administered, thus reducing the side effects. The multivalent approach is similar to that of cocktail therapy, in which a mixture of drugs is used to produce a synergistic effect. The multivalency offers a further advantage in that therapy and detection of therapeutic effects may be combined into one nanoparticle conducted under a single administration[12,50,51].

At present, a variety of other polyvalent nanoparticles have been developed; however, producing homologous particles and consistent reproduction of copy numbers within the population is challenging. Any uncertainty in structure and stoichiometry could cause unpredictable side effects or non-specific toxicity. Using RNA nanotechnology, the production of homogeneous nanoparticles can be 'manufactured' with high reproducibility, and defined structure and stoichiometry, thus facilitating quality and safety control.

The size of RNA particles on the nanometre scale is another advantage. For effective delivery to diseased tissues, many studies suggest that particles ranging from 10–50 nm are optimal for a non-viral vector because they are large enough to be retained by the body yet small enough to pass through the cell membrane by means of endocytosis, mediated by the cell-surface receptors[105]. Nanoparticle delivery has the potential to improve the pharmacokinetics, pharmacodynamics, biodistribution and safety of this newly emerging modality.

The protein-free nature will avoid the induction of antibodies, thus allowing repeated administration for the treatment of chronic diseases including cancers, viral infections and genetic ailments. Moreover, RNA nanoparticles are classified by the United States Food and Drug Administration (FDA) as chemical rather than biological entities, which will speed up the FDA approval.

The feasibility of RNA nanotechnology in disease therapy has been exemplified in the phi29 pRNA therapeutic system[14,35,50,51,106,107]. Incubation of the synthetic polyvalent RNA nanoparticles containing receptor-binding aptamers or ligands resulted in cell binding and entry of the incorporated therapeutics, subsequently modulating apoptosis[50,51]. The delivery efficiency and therapeutic effect were later confirmed in animal trials[50,51]. The 3D design, circular permutation, folding energy alteration and nucleotide modification of RNA were applied to generate RNase-resistant RNA nanoparticles with low toxicity and to ensure processing of the chimeric RNA complexes into siRNA by Dicer after delivery.

Challenges and perspectives

RNA nanoparticle construction involves conjugation of functionalities, crosslinking of modules, labelling of subunits and chemical modification of nucleotides. Methods of synthesizing RNA building blocks include both chemical and enzymatic approaches. Although great progress has been made, improvements are much needed.

Prediction of RNA structure or folding for particle assembly remains a challenge. Owing to the unusual folding properties such as non-canonical base pairing, the rules that elucidate RNA folding are yet to be sorted out. At present, using the RNA 2D prediction program by Zuker, typically only 70% of the 2D folding prediction is accurate, based on experimental data[74,75]. Clearly, predicting the RNA 3D and 4D structures is even more elusive. Computer-aided programs in RNA-structure prediction and those for computing the intermolecular interactions of RNA subunits for quaternary nanostructure formation are still to be explored.

Natural RNA is sensitive to RNase and is especially unstable in serum or in the body. This instability has long hindered its application as a construction material. Improving the stability of RNA has progressed rapidly; chemical modification of the base (for example, 5-Br-Ura and 5-I-Ura), phosphate linkage (for example, phosphothioate, boranophosphate), and/or the C2′ (for example, 2′-fluorine, 2′-O-methyl or 2′-amine)[108] have all been explored. Other attempts include peptide nucleic acids, locked nucleic acids and their respective derivatives polycarbamate nucleic acids[109] or locked nucleic acids with a bridge at different positions (2′–4′, 1′–3′)[110]. The 3′-end capping also improved the base pairing selectivity in duplex formation[111]. For all these methods, the 2′-fluorine modification is the most appraisable because it has minimal detrimental effect on RNA folding and function[112].

Loop–loop interaction is one approach to assemble quaternary RNA nanoparticles; however, dissociation of loops can occur when the concentration is reduced. Crosslinking agents, such as psoralen, nitrogen mustard derivatives and transition metal compounds[113] can promote the formation of stable RNA complexes. Recent advancements include various bifunctional agents separated by linkers and phenolic derivatives[114] to increase the efficiency of crosslinking. Long-range (> 9 Å) and short-range (1.5 Å) photoaffinity crosslinking can be achieved using azidophenacyl derivatives and thionucleosides, such as 6-thioguanosine and 4-thiouridine, respectively.

For fluorescent labelling, single conjugation of fluorophores at the 5′- or 3′-end is preferable to prevent physical hindrance. End labelling is not difficult with chemical synthesis of small RNA, however, it is challenging for long RNA requiring enzymatic methods.

To meet this challenge, guanosine monophosphate (GMP) or adenosine monophosphate (AMP) derivatives that can only be used for transcription initiation, but not for chain elongation, have been used. Fluorescent RNA can also be easily synthesized *in vitro* with T7 RNA polymerase using a new agent tCTP[115].

The challenges of *in vivo* computation using RNA[48,49] include scaling the logic operations with a large number of inputs, extending input signal types, and eliminating nonspecific actions resulting in targeting unexpected or undesired pathways.

The results of modification related to RNA folding and *in vivo* toxicity of the nucleotide derivatives remain to be explored. Owing to metabolism and biocompatibility issues, the most stable RNA might not necessarily be the most desirable; retention of particles within an appropriate time period is more attractive.

The most challenging aspect of RNA therapeutics is the yield and cost of RNA production. Commercial RNA chemical synthesis can offer only 40 (conservative) to 80 nucleotides with low yield. Acetalester 2′-OH protecting groups, such as pivaloyloxymethyl, have been reported to enhance chemical synthesis of RNA[117]. RNase ligase II has been shown to be a good alternative over the traditional T4 DNA ligase to generate longer RNA by ligation of two shorter synthetic RNA fragments[116]. In enzymatic synthesis, heterogeneity of the 3′-end has been an issue[117]; this can be addressed by extending the transcribed sequence beyond the intended end and then cleaving the RNA at the desired site using ribozymes, DNAzymes, or RNase H[116–118]. Large-scale RNA complexes produced in bacteria escorted by a tRNA vector have also been reported[40,41]. Based on the rapid reduction of cost over the history of DNA synthesis, it is expected that the cost of RNA synthesis will gradually decrease with the development of industrial-scale RNA production technologies.

In conclusion, natural or synthetic RNA molecules can fold into predefined structures that can spontaneously assemble into nanoparticles with numerous functionalities. The field of RNA nanotechnology is emerging but will play an increasingly important role in medicine, biotechnology, synthetic biology and nanotechnology.

References

1. Lin, C., Liu, Y. & Yan, H. Designer DNA nanoarchitectures. *Biochemistry* **48,** 1663–1674 (2009).
2. Aldaye, F. A., Palmer, A. L. & Sleiman, H. F. Assembling materials with DNA as the guide. *Science* **321,** 1795–1799 (2008).
3. Seeman, N. C. Nanomaterials based on DNA. *Annu. Rev. Biochem.* **79,** 65–87 (2010).
4. Moll, D. *et al.* S-layer-streptavidin fusion proteins as template for nanopatterned molecular arrays. *Proc. Natl Acad. Sci. USA* **99,** 14646–14651 (2002).
5. Cui, H., Muraoka, T., Cheetham, A. G. & Stupp, S. I. Self-assembly of giant peptide nanobelts. *Nano. Lett.* **9,** 945–951 (2009).
6. Adler-Abramovich, L. *et al.* Self-assembled arrays of peptide nanotubes by vapour deposition. *Nature Nanotech.* **4,** 849–854 (2009).
7. Knowles, T. P. *et al.* Nanostructured films from hierarchical self-assembly of amyloidogenic proteins. *Nature Nanotech.* **5,** 204–207 (2010).
8. Guo, P. *et al.* Inter-RNA interaction of phage phi29 pRNA to form a hexameric complex for viral DNA transportation. *Mol. Cell* **2,** 149–155 (1998).
9. Zhang, F. *et al.* Function of hexameric RNA in packaging of bacteriophage phi29 DNA *in vitro*. *Mol. Cell* **2,** 141–147 (1998).
10. Jaeger, L. & Leontis, N. B. Tecto-RNA: One dimensional self-assembly through tertiary interactions. *Angew. Chem. Int. Ed.* **39,** 2521–2524 (2000).
11. Jaeger, L., Westhof, E. & Leontis, N. B. TectoRNA: modular assembly units for the construction of RNA nano-objects. *Nucleic Acids Res.* **29,** 455–463 (2001).
12. Shu, D. *et al.* Bottom-up assembly of RNA arrays and superstructures as potential parts in nanotechnology. *Nano Lett.* **4,** 1717–1723 (2004).
13. Chworos, A. *et al.* Building programmable jigsaw puzzles with RNA. *Science* **306,** 2068–2072 (2004).
14. Guo, P. RNA nanotechnology: Engineering, assembly and applications in detection, gene delivery and therapy. *J. Nanosci. Nanotechnol.* **5,** 1964–1982 (2005).
15. Jaeger, L. & Chworos, A. The architectonics of programmable RNA and DNA nanostructures. *Curr. Opin. Struct. Biol.* **16,** 531–543 (2006).
16. Ikawa, Y., Tsuda, K., Matsumura, S. & Inoue, T. *De novo* synthesis and development of an RNA enzyme. *Proc. Natl Acad. Sci. USA* **101,** 13750–13755 (2004).
17. Matsumura, S. *et al.* Coordinated control of a designed trans-acting ligase ribozyme by a loop-receptor interaction. *FEBS Lett.* **583,** 2819–2826 (2009).
18. Leontis, N. B., Lescoute, A. & Westhof, E. The building blocks and motifs of RNA architecture. *Curr. Opin. Struct. Biol.* **16,** 279–287 (2006).
19. Schroeder, K. T., McPhee, S. A., Ouellet, J. & Lilley, D. M. A structural database for k-turn motifs in RNA. *RNA* **16,** 1463–1468 (2010).
20. Li, X., Horiya, S. & Harada, K. An efficient thermally induced RNA conformational switch as a framework for the functionalization of RNA nanostructures. *J. Am. Chem. Soc.* **128,** 4035–4040 (2006).
21. Nasalean, L., Baudrey, S., Leontis, N. B. & Jaeger, L. Controlling RNA self-assembly to form filaments. *Nucleic Acids Res.* **34,** 1381–1392 (2006).
22. Liu, B., Baudrey, S., Jaeger, L. & Bazan, G. C. Characterization of tectoRNA assembly with cationic conjugated polymers. *J. Am. Chem. Soc.* **126,** 4076–4077 (2004).
23. Cayrol, B. *et al.* A nanostructure made of a bacterial noncoding RNA. *J. Am. Chem. Soc.* **131,** 17270–17276 (2009).
24. Geary, C., Chworos, A. & Jaeger, L. Promoting RNA helical stacking via A-minor junctions. *Nucleic Acids Res.* doi:10.1093/nar/gkq748 (2010).
25. Sugimoto, N. *et al.* Thermodynamic parameters to predict stability of RNA/DNA hybrid duplexes. *Biochemistry* **34,** 11211–11216 (1995).
26. Searle, M. S. & Williams, D. H. On the stability of nucleic acid structures in solution: enthalpy-entropy compensations, internal rotations and reversibility. *Nucleic Acids Res.* **21,** 2051–2056 (1993).
27. Kitamura, A. *et al.* Analysis of intermolecular base pair formation of prohead RNA of the phage phi29 DNA packaging motor using NMR spectroscopy. *Nucleic Acids Res.* **36,** 839–848 (2008).
28. Chen, C., Zhang, C. & Guo, P. Sequence requirement for hand-in-hand interaction in formation of pRNA dimers and hexamers to gear phi29 DNA translocation motor. *RNA* **5,** 805–818 (1999).
29. Severcan, I. *et al.* Square-shaped RNA particles from different RNA folds. *Nano Lett.* **9,** 1270–1277 (2009).
30. Severcan, I. *et al.* A polyhedron made of tRNAs. *Nature Chem.* **2,** 772–779 (2010).
31. Hansma, H. G., Oroudjev, E., Baudrey, S. & Jaeger, L. TectoRNA and 'kissing-loop' RNA: atomic force microscopy of self-assembling RNA structures. *J. Microsc.* **212,** 273–279 (2003).
32. Lee, R. J., Wang, S. & Low, P. S. Measurement of endosome pH following folate receptor-mediated endocytosis. *Biochim. Biophys. Acta* **1312,** 237–242 (1996).
33. Pogocki, D. & Schoneich, C. Chemical stability of nucleic acid-derived drugs. *J. Pharm. Sci.* **89,** 443–456 (2000).
34. Laurenti, E. *et al.* Inducible gene and shRNA expression in resident hematopoietic stem cells *in vivo*. *Stem Cells* **28,** 1390–1398 (2010).
35. Hoeprich, S. *et al.* Bacterial virus phi29 pRNA as a hammerhead ribozyme escort to destroy hepatitis B virus. *Gene Ther.* **10,** 1258–1267 (2003).
36. Chang, K. Y. & Tinoco, I. Jr Characterization of a "kissing" hairpin complex derived from the human immunodeficiency virus genome. *Proc. Natl Acad. Sci. USA* **91,** 8705–8709 (1994).
37. Bindewald, E. *et al.* RNAJunction: a database of RNA junctions and kissing loops for three-dimensional structural analysis and nanodesign. *Nucleic Acids Res.* **36**, D392–D397 (2008).
38. Wagner, C., Ehresmann, C., Ehresmann, B. & Brunel, C. Mechanism of dimerization of bicoid mRNA: initiation and stabilization. *J. Biol. Chem.* **279,** 4560–4569 (2004).
39. Chen, C., Sheng, S., Shao, Z. & Guo, P. A dimer as a building block in assembling RNA: A hexamer that gears bacterial virus phi29 DNA-translocating machinery. *J. Biol. Chem.* **275,** 17510–17516 (2000).
40. Ponchon, L., Beauvais, G., Nonin-Lecomte, S. & Dardel, F. A generic protocol for the expression and purification of recombinant RNA in Escherichia coli using a tRNA scaffold. *Nature Protoc.* **4,** 947–959 (2009).
41. Kuwabara, T. *et al.* Formation of a catalytically active dimer by tRNA-driven short ribozymes. *Nature Biotechnol.* **16,** 961–965 (1998).
42. Fire, A. *et al.* Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* **391,** 806–811 (1998).
43. Li, H., Li, W. X. & Ding, S. W. Induction and suppression of RNA silencing by an animal virus. *Science* **296,** 1319–1321 (2002).
44. Breaker, R. R. Complex riboswitches. *Science* **319,** 1795–1797 (2008).
45. Fabian, M. R., Sonenberg, N. & Filipowicz, W. Regulation of mRNA translation and stability by microRNAs. *Annu. Rev. Biochem.* **79,** 351–379 (2010).
46. Zhang, C. Novel functions for small RNA molecules. *Curr. Opin. Mol. Ther.* **11,** 641–651 (2009).
47. Marvin, M. C. & Engelke, D. R. Broadening the mission of an RNA enzyme. *J. Cell Biochem.* **108,** 1244–1251 (2009).
48. Benenson, Y. RNA-based computation in live cells. *Curr. Opin. Biotechnol.* **20,** 471–478 (2009).

49. Shlyakhtenko, L. S. *et al.* Silatrane-based surface chemistry for immobilization of DNA, protein-DNA complexes and other biological materials. *Ultramicroscopy* **97,** 279–287 (2003).
50. Guo, S., Tschammer, N., Mohammed, S. & Guo, P. Specific delivery of therapeutic RNAs to cancer cells via the dimerization mechanism of phi29 motor pRNA. *Hum. Gene Ther.* **16,** 1097–1109 (2005).
51. Khaled, A., Guo, S., Li, F. & Guo, P. Controllable self-assembly of nanoparticles for specific delivery of multiple therapeutic molecules to cancer cells using RNA nanotechnology. *Nano Lett.* **5,** 1797–1808 (2005).
52. Shu, D., Huang, L., Hoeprich, S. & Guo, P. Construction of phi29 DNA-packaging RNA (pRNA) monomers, dimers and trimers with variable sizes and shapes as potential parts for nano-devices. *J. Nanosci. Nanotechnol.* **3,** 295–302 (2003).
53. Turner, R. & Tijan, R. Leucine repeats and an adjacent DNA binding domain mediate the formation of functional c-Fos and c-Jun heterodimers. *Science* **243,** 1689–1694 (1989).
54. Liu, D. *et al.* Tensegrity: construction of rigid DNA triangles with flexible four-arm DNA junctions. *J. Am. Chem. Soc.* **126,** 2324–2325 (2004).
55. Rothemund, P. W., Papadakis, N. & Winfree, E. Algorithmic self-assembly of DNA Sierpinski triangles. *PLoS. Biol.* **2,** e424 (2004).
56. Park, S. H. *et al.* Three-helix bundle DNA tiles self-assemble into 2D lattice or 1D templates for silver nanowires. *Nano Lett.* **5,** 693–696 (2005).
57. Weizmann, Y. *et al.* A polycatenated DNA scaffold for the one-step assembly of hierarchical nanostructures. *Proc. Natl Acad. Sci. USA* **105,** 5289–5294 (2008).
58. Aldaye, F. A. & Sleiman, H. F. Modular access to structurally switchable 3D discrete DNA assemblies. *J. Am. Chem. Soc.* **129,** 13376–13377 (2007).
59. Zheng, J. *et al.* From molecular to macroscopic via the rational design of a self-assembled 3D DNA crystal. *Nature* **461,** 74–77 (2009).
60. Rothemund, P. W. K. Folding DNA to create nanoscale shapes and patterns. *Nature* **440,** 297–302 (2006).
61. Andersen, E. S. *et al.* Self-assembly of a nanoscale DNA box with a controllable lid. *Nature* **459,** 73–76 (2009).
62. Ke, Y. G. *et al.* Self-assembled water-soluble nucleic acid probe tiles for label-free RNA hybridization assays. *Science* **319,** 180–183 (2008).
63. Douglas, S. M., Chou, J. J. & Shih, W. M. DNA-nanotube-induced alignment of membrane proteins for NMR structure determination. *Proc. Natl Acad. Sci. USA* **104,** 6644–6648 (2007).
64. Endo, M., Seeman, N. C. & Majima, T. DNA tube structures controlled by a four-way-branched DNA connector. *Angew. Chem. Int. Ed. Engl.* **44,** 6074–6077 (2005).
65. Tanaka, K. *et al.* A discrete self-assembled metal array in artificial DNA. *Science* **299,** 1212–1213 (2003).
66. Yurke, B. *et al.* A DNA-fuelled molecular machine made of DNA. *Nature* **406,** 605–608 (2000).
67. Lin, C. *et al. In vivo* cloning of artificial DNA nanostructures. *Proc. Natl Acad. Sci. USA* **105,** 17626–17631 (2008).
68. Eckardt, L. H. *et al.* DNA nanotechnology: Chemical copying of connectivity. *Nature* **420,** 286 (2002).
69. Endo, M. *et al.* Programmed-assembly system using DNA jigsaw pieces. *Chemistry* **16,** 5362–5368 (2010).
70. Dietz, H., Douglas, S. M. & Shih, W. M. Folding DNA into twisted and curved nanoscale shapes. *Science* **325,** 725–730 (2009).
71. Cherny, D. I., Eperon, I. C. & Bagshaw, C. R. Probing complexes with single fluorophores: factors contributing to dispersion of FRET in DNA/RNA duplexes. *Eur. Biophys. J.* **38,** 395–405 (2009).
72. Shapiro, B. A. Computational design strategies for RNA nanostructures. *J. Biomol. Struct. Dyn.* **26,** 820 (2009).
73. Yingling, Y. G. & Shapiro, B. A. Computational design of an RNA hexagonal nanoring and an RNA nanotube. *Nano Lett.* **7,** 2328–2334 (2007).
74. Zuker, M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* **31,** 3406–3415 (2003).
75. Markham, N. R. & Zuker, M. UNAFold: software for nucleic acid folding and hybridization. *Methods Mol. Biol.* **453,** 3–31 (2008).
76. Bindewald, E. *et al.* Computational strategies for the automated design of RNA nanoscale structures from building blocks using NanoTiler. *J. Mol. Graph. Model.* **27,** 299–308 (2008).
77. Afonin, K. A. *et al. In vitro* assembly of cubic RNA-based scaffolds designed *in silico. Nature Nanotech.* **5,** 676–682 (2010).
78. Chakraborty, S., Modi, S. & Krishnan, Y. The RNA2-PNA2 hybrid i-motif-a novel RNA-based building block. *Chem. Commun.* 70–72 (2008).
79. Afonin, K. A., Cieply, D. J. & Leontis, N. B. Specific RNA self-assembly with minimal paranemic motifs. *J. Am. Chem. Soc.* **130,** 93–102 (2008).
80. Lescoute, A. & Westhof, E. Topology of three-way junctions in folded RNAs. *RNA* **12,** 83–93 (2006).
81. Ouellet, J. *et al.* Structure of the three-way helical junction of the hepatitis C virus IRES element. *RNA* **16,** 1597–1609 (2010).
82. de la, P. M., Dufour, D. & Gallego, J. Three-way RNA junctions with remote tertiary contacts: a recurrent and highly versatile fold. *RNA* **15,** 1949–1964 (2009).
83. Griffiths-Jones, S. *et al.* Rfam: annotating non-coding RNAs in complete genomes. *Nucleic Acids Res.* **33,** D121–D124 (2005).
84. Abraham, M., Dror, O., Nussinov, R. & Wolfson, H. J. Analysis and classification of RNA tertiary structures. *RNA* **14,** 2274–2289 (2008).
85. Guo, P., Erickson, S. & Anderson, D. A small viral RNA is required for *in vitro* packaging of bacteriophage phi29 DNA. *Science* **236,** 690–694 (1987).
86. Xiao, F., Demeler, B. & Guo, P. Assembly mechanism of the sixty-subunit nanoparticles via interaction of RNA with the reengineered protein connector of phi29 DNA-packaging motor. *ACS Nano* **4,** 3293–3301 (2010).
87. Shu, D., Zhang, H., Jin, J. & Guo, P. Counting of six pRNAs of phi29 DNA-packaging motor with customized single molecule dual-view system. *EMBO J.* **26,** 527–537 (2007).
88. Woodson, S. A. Compact intermediates in RNA folding. *Annu. Rev. Biophys.* **39,** 61–77 (2010).
89. Gugliotti, L. A., Feldheim, D. L. & Eaton, B. E. RNA-mediated metal-metal bond formation in the synthesis of hexagonal palladium nanoparticles. *Science* **304,** 850–852 (2004).
90. Koyfman, A. Y. *et al.* Controlled spacing of cationic gold nanoparticles by nanocrown RNA. *J. Am. Chem. Soc.* **127,** 11886–11887 (2005).
91. Oguro, A., Ohtsu, T. & Nakamura, Y. An aptamer-based biosensor for mammalian initiation factor eukaryotic initiation factor 4A. *Anal. Biochem.* **388,** 102–107 (2009).
92. Mi, J. *et al. In vivo* selection of tumor-targeting RNA motifs. *Nature Chem. Biol.* **6,** 22–24 (2010).
93. Liu, Y. *et al.* Targeting hypoxia-inducible factor-1alpha with Tf-PEI-shRNA complex via transferring receptor-mediated endocytosis inhibits melanoma growth. *Mol. Ther.* **17,** 269–277 (2009).
94. Kumar, P. *et al.* Transvascular delivery of small interfering RNA to the central nervous system. *Nature* **448,** 39–43 (2007).
95. Kruger, K. *et al.* Self-splicing RNA: autoexcision and autocyclization of the ribosomal RNA intervening sequence of Tetrahymena. *Cell* **31,** 147–157 (1982).
96. Guerrier-Takada, C. *et al.* The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. *Cell* **35,** 849–857 (1983).
97. Ellington, A. D. & Szostak, J. W. *In vitro* selection of RNA molecules that bind specific ligands. *Nature* **346,** 818–822 (1990).
98. Tuerk, C. & Gold, L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA ploymerase. *Science* **249,** 505–510 (1990).
99. Ellington, A. D. Back to the future of nucleic acid self-amplification. *Nature Chem. Biol.* **5,** 200–201 (2009).
100. Zhou, J., Li, H., Zaia, J. & Rossi, J. J. Novel dual inhibitory function aptamer-siRNA delivery system for HIV-1 therapy. *Mol. Ther.* **16,** 1481–1489 (2008).
101. Bunka, D. H. *et al.* Production and characterization of RNA aptamers specific for amyloid fibril epitopes. *J. Biol. Chem.* **282,** 34500–34509 (2007).
102. Sudarsan, N. *et al.* Riboswitches in eubacteria sense the second messenger cyclic di-GMP. *Science* **321,** 411–413 (2008).
103. Ogawa, A. & Maeda, M. An artificial aptazyme-based riboswitch and its cascading system in E. coli. *Chembiochem* **9,** 206–209 (2008).
104. Shahbabian, K., Jamalli, A., Zig, L. & Putzer, H. RNase Y, a novel endoribonuclease, initiates riboswitch turnover in Bacillus subtilis. *EMBO J.* **28,** 3523–3533 (2009).
105. Prabha, S., Zhou, W. Z., Panyam, J. & Labhasetwar, V. Size-dependency of nanoparticle-mediated gene transfection: studies with fractionated nanoparticles. *Int. J. Pharm.* **244,** 105–115 (2002).
106. Guo, S., Huang, F. & Guo, P. Construction of folate-conjugated pRNA of bacteriophage phi29 DNA packaging motor for delivery of chimeric siRNA to nasopharyngeal carcinoma cells. *Gene Ther.* **13,** 814–820 (2006).
107. Zhang, H. M. *et al.* Target delivery of anti-coxsachievirus siRNAs using ligand-conjugated packaging RNAs. *Antivir. Res.* **83,** 307–316 (2009).
108. Watts, J. K., Deleavey, G. F. & Damha, M. J. Chemically modified siRNA: tools and applications. *Drug Discov. Today* **13,** 842–855 (2008).
109. Madhuri, V. & Kumar, V. A. Design, synthesis and DNA/RNA binding studies of nucleic acids comprising stereoregular and acyclic polycarbamate backbone: polycarbamate nucleic acids (PCNA). *Org. Biomol. Chem.* **8,** 3734–3741 (2010).
110. Mathe, C. & Perigaud, C. Recent approaches in the synthesis of conformationally restricted nucleoside analogues. *Eur. J. Org. Chem.* 1489–1505 (2008).
111. Patra, A. & Richert, C. High fidelity base pairing at the 3′-terminus. *J. Am. Chem. Soc.* **131,** 12671–12681 (2009).

112. Liu, J. *et al.* Fabrication of stable and RNase-resistant RNA nanoparticles active in gearing the nanomotors for viral DNA packaging. *ACS Nano* (in the press).
113. Efimov, V. A., Fediunin, S. V. & Chakhmakhcheva, O. G. Cross-linked nucleic acids: formation, structure, and biological function. *Bioorg. Khim.* **36,** 56–80 (2010).
114. Song, Z. *et al.* Synthesis and oxidation-induced DNA cross-linking capabilities of bis(catechol) quaternary ammonium derivatives. *Chemistry* **14,** 5751–5754 (2008).
115. Stengel, G., Urban, M., Purse, B. W. & Kuchta, R. D. Incorporation of the fluorescent ribonucleotide analogue tCTP by T7 RNA polymerase. *Anal. Chem.* **82,** 1082–1089 (2010).
116. Solomatin, S. & Herschlag, D. Methods of site-specific labeling of RNA with fluorescent dyes. *Methods Enzymol.* **469,** 47–68 (2009).
117. Lavergne, T., Bertrand, J. R., Vasseur, J. J. & Debart, F. A base-labile group for 2′-OH protection of ribonucleosides: a major challenge for RNA synthesis. *Chemistry* **14,** 9135–9138 (2008).
118. Hoeprich, S. & Guo, P. Computer modeling of three-dimensional structure of DNA-packaging RNA(pRNA) monomer, dimer, and hexamer of phi29 DNA packaging motor. *J. Biol. Chem.* **277,** 20794–20803 (2002).

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Additional information

The author declares competing financial interests: details accompany the paper at www.nature.com/naturenanotechnology.