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The architectonics of programmable RNA and DNA nanostructures

Luc Jaeger and Arkadiusz Chworos

The past several years have witnessed the emergence of a new world of nucleic-acid-based architectures with highly predictable and programmable self-assembly properties. For almost two decades, DNA has been the primary material for nucleic acid nanoconstruction. More recently, the dramatic increase in RNA structural information led to the development of RNA architectonics, the scientific study of the principles of RNA architecture with the aim of constructing RNA nanostructures of any arbitrary size and shape. The remarkable modularity and the distinct but complementary nature of RNA and DNA nanomaterials are revealed by the various self-assembly strategies that aim to achieve control of the arrangement of matter at a nanoscale level.

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Introduction

The complex supramolecular (see glossary) structures that emerged in living organisms through billions of years of evolution rely on two basic self-assembly processes: the spontaneous folding of one polymer chain into a stable well-defined 3D structure; and the assembly of multiple subunits into defined, modular supramolecular architectures. Key characteristics are hierarchical organization, modular components, and stereochemically specific and selective interactions. Programmable assembly (see glossary) results from the application of folding and assembly principles gleaned from biological structures to design molecules that will, in a predictable manner, fold into specific shapes and subsequently assemble with one another into supramolecular architectures according to the structural information encoded within their primary structure. Although programmable self-assembly is at the core of supramolecular chemistry [1], it reaches a more complex dimension with proteins and nucleic acids. Proteins are the material of choice for building the structural, catalytic and regulatory components of cells, but their folding and assembly remain challenging to predict and design because of the inherent complexity of their 3D structures (see the reviews from Ranganathan, Waters, Kuhlman and Chin in this issue). By contrast, DNA, as the carrier of the genetic information in cells, has only four deoxynucleotide chemical building blocks, a high chemical stability, and predictable folding and assembly properties that are readily amenable to the rational design and construction of 3D nanostructures by programmable self-assembly [2,3,4•–6•]. RNA has recently emerged as a challenger to DNA, interesting in its own right as a medium for programmable nanoconstruction (e.g. [7,8,9••,10,11•,12•]). Despite a chemical structure very similar to that of DNA, RNA is chemically more labile than DNA, but is also more prone to fold into complex tertiary structures with recognition and catalytic properties reminiscent of those of proteins. Natural RNAs are the working components of biologically important molecular machines that are capable of using cellular energy in the form of ATP or GTP to perform mechanical work and to carry out complex tasks of information processing, such as template-directed protein synthesis and multiplexed gene regulation [13]. If one can argue that this greater functional versatility of RNA versus DNA in nature results from historical evolutionary contingencies, it is nonetheless apparent that the RNA tertiary folding and assembly principles that are currently emerging from the analysis of NMR and crystallographic structures of RNAs [14,15] are significantly different from those of DNA (e.g. [16,17]) and offer new possibilities for the rational design of complex nanoarchitectures [18] (Figure 1).

Several interesting reviews have been recently dedicated to DNA-based nanostructures from the rational design, chemical and nanotechnological point of view [2,3,4•–6•]. Herein, we place a stronger emphasis on nanostructures made of RNA, and introduce the architectonics (see glossary) of RNA and DNA as the scientific study of nucleic-acid-based architecture. This field of investigation encompasses the principles of the design, construction and ornamentation (or functionalization) of useful and fine nanostructures made of nucleic acid materials.

Basic structural properties and modularity of RNA and DNA nanostructures

RNA and DNA modularity is hierarchically expressed at a chemical, structural and supramolecular level (Figure 1). Within this hierarchical framework, stacking and

Glossary

Addressable: characteristic of a supramolecular architecture whereby the final position of each constitutive molecular unit can be known without ambiguity within the assembly.

Aptamer: an oligonucleotide that folds into a structure that is able to specifically recognize and bind a ligand.

Assembling interfaces: formed by 4° interactions between interacting cohesive edges of two adjacent tile or tectoRNA units.

Nucleic acid architectonics: the scientific study of nucleic acid architecture.

Programmable: characteristic of a molecule or ensemble of molecules whereby the information specified at the sequence level can be controlled with high predictability to fold and assemble into predefined 3D architectures.

Ribozyme: RNA sequence or domain with catalytic chemical properties.

RNA tectonics: the modular character of RNA molecules that can be decomposed and reassembled into new nanoscopic architectures.

Secondary (2°) and tertiary (3°) structure motifs: recurrent and specific sets of nucleotides that reproducibly form unique 2° and/or 3° structures. 2° structure motifs are stable helical regions that specify the formation of loops, internal loops, bulges and multihelix junctions. 3° structure motifs are unique stable 3D conformers. Whereas 2° structure motifs can act as flexible hinges, 3° structure motifs are rigid structural elements stabilized by 3° interactions between specific nucleotide positions. 3° interactions can be mediated by non-classic Watson–Crick base pairing, also called non-canonical base pairing. A 3° structure motif always corresponds to a 2° structure motif, but the opposite is not always true.

siRNA: small non-coding RNA molecules that mediate the silencing of particular genes by an RNA interference mechanism [13].

Supramolecular: characteristic of an assembly of multiple molecules formed through non-covalent interactions.

TectoRNA: a basic non-irreducible RNA molecular unit that forms RNA architectures.

Tile motif: rigid structural building block with assembling interfaces that can form larger periodic or aperiodic nanostructures. Tiles are often generated by supramolecular assembly of several DNA strands or tectoRNA units.

Wang tiles: In the mathematical theory of tiling, Wang tiles are defined as 2D geometrical shapes with colored edges that can only assemble between edges of the same color. Assembly of Wang tiles can be used to simulate the operation of a chosen Turing machine. For more information, see references in [32,61•].

Watson–Crick base pairing between complementary nucleotides drive the folding and assembly of RNA and DNA primary (1°) sequences into secondary (2°) structures (see glossary) through the formation of helical elements that define hairpin loops, bulges, internal loops and multihelix junctions (Figure 1). As basic modular building blocks, A-form RNA helices are more compact, stiffer [19,20] and thermodynamically more stable than B-form DNA helices [21,22] (Figure 1). Additionally, non-canonical base pairs can contribute significantly to the rigidity and thermodynamic stability of RNA structural elements [23]. The design and prediction of RNA and DNA 2° structures can presently be achieved by energy minimization with a reasonable degree of accuracy [23,24]. However, because the formation of mismatches between non-perfectly complementary strands is allowed, RNA helices have a lower selective information content than their DNA counterparts. Thus, for RNA, positive and negative design is particularly critical to maximize the stability of the desired 2° structure while minimizing folding into stable alternative 2° structures.

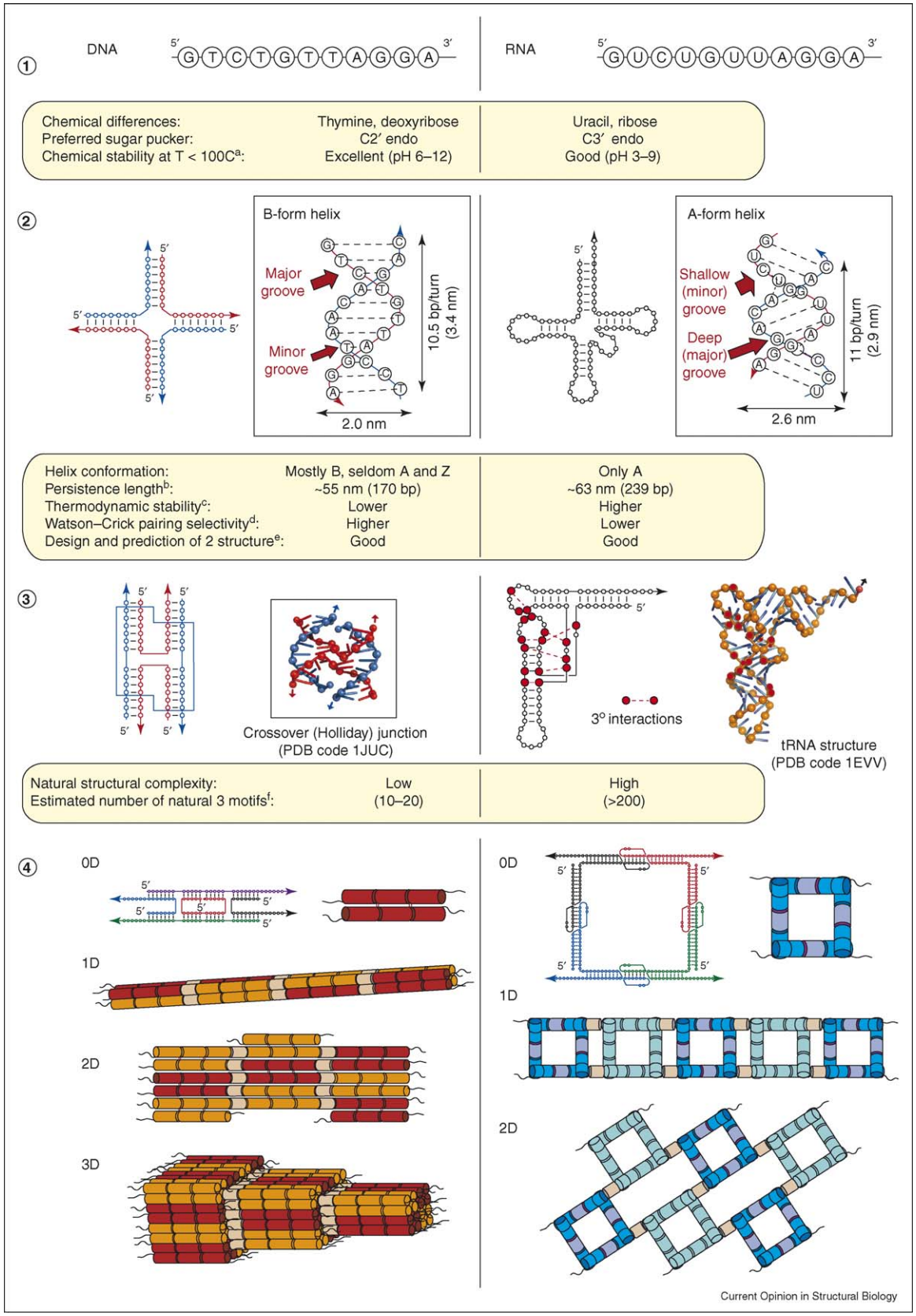
Despite a limited number of known DNA tertiary (3°) structure motifs (see glossary; Figure 2a), the remarkable base-pairing selectivity of DNA is particularly well suited to building self-assembling architectures that predominantly rely on the selective formation of 2° structure elements between multiple complementary oligonucleotide strands (Figure 1). By contrast, the architectural potential of RNA relies more on the ability of an RNA single strand to fold into an exquisite, stable 3° structure [18]. At this level of organization, the 2° structure elements can associate through numerous van der Waals contacts, π stacking, metal coordination and specific hydrogen bonds via the formation of a small number of additional Watson–Crick and/or non-Watson–Crick base pairs involving single-stranded regions, loops or bulges. Natural RNAs offer a rich treasure-trove of recurrent and modular 3° structure motifs, which have been identified by data mining known NMR and crystallographic atomic structures [14,15]. These motifs specify a precise geometry of helical elements, and mediate stereochemically precise and readily reversible 3° and quaternary (4°) interactions (Figure 2b). Thus, rather than relying solely on 2° structure elements, the 3° structure of an RNA can be engineered by encoding the structural information corresponding to rigid 3° structure motifs within its sequence. The separation of energy levels between 2° and 3° structures is distinct for stable natural RNAs, with 2° structure elements being more stable than 3° elements [13]. For a complex RNA, the dependence of the 3° structure on the presence of the extended and correct 2° structure might therefore be a necessity to avoid kinetically trapped misfolded states.

At a 4° structure level, RNA and DNA modular units assemble further into complex and highly modular supramolecular architectures in a predictable manner using base-pair rules as organizational instructions. The dimensionality of these nanostructures is directly related to the number, shape, geometry and orientation of cohesive, assembling interfaces formed between constitutive RNA or DNA tiles (see glossary) [6•] (Figure 1).

DNA architectonics: variations on the same structural theme

Because of the lack of stable natural 3° structure motifs, much effort has been expended designing robust and rigid DNA self-assembling building blocks [2]. All engineered DNA 'tiles' are essentially formed using a small number of structural rules derived from crossover (Holliday) junction motifs (Figure 1) [25]. They are typically assembled from multiple oligonucleotide strands that interact through selective complementary Watson–Crick base pairing and intertwine through crossover motifs (Figure 2d) (e.g. [26,27,28•,29–36,37•,38–40]).

Figure 1


①
DNA
5′ G T C T G T T A G G A 3′
RNA
5′ G U C U G U U A G G A 3′
Chemical differences: Thymine, deoxyribose | Uracil, ribose
Preferred sugar pucker: C2′ endo | C3′ endo
Chemical stability at T < 100C[a]: Excellent (pH 6–12) | Good (pH 3–9)
②
B-form helix
Major groove
Minor groove
10.5 bp/turn (3.4 nm)
2.0 nm
A-form helix
Shallow (minor) groove
Deep (major) groove
11 bp/turn (2.9 nm)
2.6 nm
Helix conformation: Mostly B, seldom A and Z | Only A
Persistence length[b]: ~55 nm (170 bp) | ~63 nm (239 bp)
Thermodynamic stability[c]: Lower | Higher
Watson–Crick pairing selectivity[d]: Higher | Lower
Design and prediction of 2 structure[e]: Good | Good
③
Crossover (Holliday) junction (PDB code 1JUC)
3° interactions
tRNA structure (PDB code 1EVV)
Natural structural complexity: Low | High
Estimated number of natural 3 motifs[f]: (10–20) | (>200)
④
0D
1D
2D
3D
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In particular, the design of robust helical-bundle tiles [36,37•,38–40] offers an attractive framework for generating 1D, 2D or 3D nanostructures through fine-tuning of the positioning of crossover motifs that join parallel helical stacks (Figures 1 and 2d) [41]. As multimolecular assemblies, DNA tiles can readily be considered 4° structures, but from structural and thermodynamic stand points, no clear distinction can be established between their 2°, 3° and 4° structures, because their formation is essentially based on 2° structure constraints (Figure 1). As such, DNA architectures are less hierarchical than those of RNA and assemble by strand invasion processes similar to those operating during homologous recombination events.

A subtle balance of flexibility and stress is required for building good self-assembling tiles [31], but stable rigid 3° structure motifs are not an absolute requirement. The vertices of triangulated architectures can be flexible as triangulated structures should be able to resist deformation through tensegrity, a geometric construction principle that combines stiff helical struts that push outward and flexible junctions that push inward (Figure 2c). By taking advantage of this principle, stable triangular DNA tiles that are able to assemble into extensive Kagome-like lattices [27,28•], a replicable octahedron cage [42••] and a rigid tetrahedron building block [43•] have been recently built.

The monolithic structure of most DNA tiles imposes strong geometrical constraints on the positioning of their cohesive interfaces (Figure 2d). Typically, only a reduced number of different 4° supramolecular architectures can be generated from a particular design of tile. DNA cohesive interfaces are typically formed through complementary Watson–Crick base pairing between collinear tail connectors of adjacent tiles [32] (Figure 2a). They can also occur through formation of paranemic crossovers between internal loops that are wrapped around one another and do not interpenetrate topologically [44] (Figure 2a). Variation in the number of tail connectors and their thermodynamic stability can be used to modulate the assembly process as a function of temperature, DNA molecules and salt concentration.

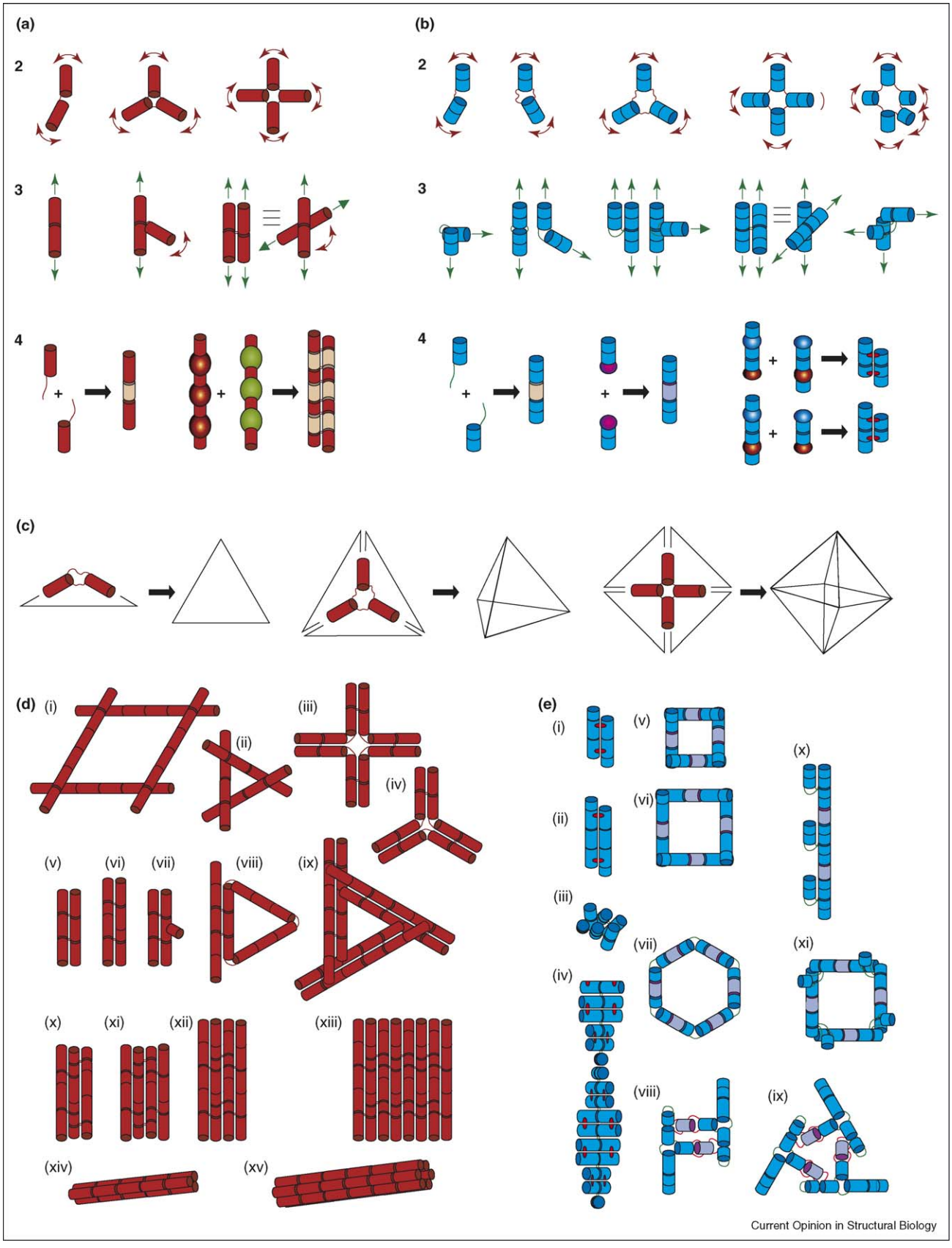
In the future, the use of triple helices [17], G-tetrads [16] and non-Watson–Crick parallel strands [45] will probably expand the modes of assembly of DNA tiles. Moreover, considering that DNA can fold into stable aptamers (see glossary), the full potential of DNA 3° structure for nanoconstruction has clearly not been exploited yet. However, the real potential of DNA lies more in the optimal use of its simple rules of assembly, based on the unique selectivity of Watson–Crick base pairing, rather than its 3° structure diversity, as exemplified by the recent development of scaffolded DNA origami [46••], discussed later in this review.

RNA architectonics: sculpting new RNA structures

The concept of RNA tectonics (see glossary) was initially defined as referring to the modular character of RNA structures that can be decomposed and reassembled to create new modular RNA units, called tectoRNAs (see glossary), which are able to self-assemble into nanoscale and mesoscale architectures of any desired size and shape [18]. RNA architectonics, the science behind this concept, is grounded in the methodological approach described in Figure 3 [9••,12•,47]. RNA is readily amenable to inverse folding: supramolecular models can be 'sketched' in 3D space by positioning the modular 3° structure motifs and 4° intermolecular interactions that define the geometry and the self-assembling interfaces necessary to create the desired nanoscale architecture, and then connecting the motifs using semi-rigid double-helical 'struts'. Because of the large variety of known modular and pre-organized 3° structure motifs [14,15], the

(Figure 1 Legend) Chemical, structural and supramolecular modularity of RNA and DNA. RNA and DNA chemical modularity is exemplified by their 1° sequence. The structural and conformational modularity of nucleic acids is expressed at the level of their 2° and 3° structures. Helices, comprising modular isosteric base pairs, are the basic building blocks that form the 2° structure of RNA and DNA. RNA and, to a much lesser extent, DNA are also made of recurrent motifs that shape their 3° structure [14,15]. Modularity is also expressed at the supramolecular level: RNA and DNA units can be engineered to assemble into highly modular 4° architectures with different dimensionalities [6•]. The dimensionality of a supramolecular object can be defined in terms of modularity, spatial arrangement of constitutive units and vectorial assembly growth. Objects of dimensionality zero (0D) are supramolecular architectures of finite size that can best be described as non-reducible modular tiles. They are modular, but are formed of distinct non-repetitive units. Objects of dimensionality one (1D), two (2D) and three (3D) are characterized by units with at least two, three or four assembling interfaces, directing assembly in 1D, 2D or 3D in Cartesian space, respectively. [a]At basic pH, the RNA backbone hydrolyzes in the presence of divalent ions such as magnesium. RNA is also easily degraded by ribonucleases. At acidic pH, however, DNA depurinates faster than RNA. [b]The persistence lengths of RNA and DNA were determined experimentally by single-molecule analysis (e.g. [19,20]). [c]According to the base-pair free energy parameters determined at 1 M NaCl and 37 °C for RNA and DNA, RNA base pairs are, on average, -0.49 ± 0.35 kcal/mol more stable than DNA ones [21,22]. Note, however, that the thermodynamic stability of RNA and DNA duplexes varies as a function of the nucleic acid sequence. [d]In contrast to DNA, 'non-canonical' base pairs can contribute significantly to the stability of duplexes in RNA. Thus, discrimination between perfect, complementary Watson–Crick duplexes and mismatched duplexes is better for DNA. [e]Design and prediction of the 2° structures of RNA and DNA can be achieved by free energy minimization with good accuracy [23]. The design and prediction of nucleic acid 3° nanostructures can be achieved with computer modeling using graphical user interfaces. DNA 3° structures are essentially based on a very limited number of structural rules derived from the crossover motif [25]. Albeit structurally much more complex, the design and prediction of RNA 3° structures can be successfully achieved by precise 3D modeling using X-ray or NMR atomic structural information [9••,12•]. [f]The estimated number of natural 3° structure motifs is based on a comparison of the available crystallographic structures of DNA and RNA (L Jaeger, unpublished).

Figure 2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(vi)
(vii)
(viii)
(ix)
(x)
(xi)
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number of possible architectures that can be designed using this mosaic modeling process is limitless [18]. In a second step, a 2° structure diagram corresponding to a particular 3D model of tectoRNA is used as a blueprint for the rational design of several 1° sequences expected to fold and assemble according to the initial sketched 3° and 4° model prediction (Figure 3).

The characterization of tectoRNA folding and self-assembly properties is typically performed by biochemical and biophysical methods, and visualization techniques, such as atomic force microscopy (AFM) [9••,47] and transmission electron microscopy (TEM) [12•]. The effect and contribution of specific 3° structure motifs to the overall geometry and stability of the resulting supramolecular architecture can be assessed by introducing sequence mutations at key 3° nucleotide positions within tectoRNA molecules (e.g. [7,9••,48,49]). Mutated tectoRNA assemblies are used as negative control for comparison to non-mutated ones. Thus, this approach can also be a powerful way to unravel the structural properties of 3° and 4° structure motifs for which little experimental data are available.

Although still a new field of investigation, RNA architectonics has already generated a great variety of tectoRNA units able to assemble into highly modular supramolecular architectures of arbitrary shapes (Figures 1, 2 and 4). Besides classic cohesive Watson–Crick base pairing, the formation of long-range RNA–RNA interactions, such as loop–receptor and loop–loop interactions, offers a wide range of 4° intermolecular interfaces with various thermodynamic strengths to promote assembly under the cooperative dependency of divalent ions (e.g. [7,9••,12•,50]) (Figure 2). In the presence of magnesium, kissing loop motifs are more stable than RNA duplexes with identical sequences by two or three orders of magnitude [9••,50]. Moreover, the dynamic equilibrium of assembly through 4° RNA interfaces can be tuned over four to five orders of magnitude by adjusting the magnesium ion concentration and temperature. Thus, the hierarchical self-assembly of tectoRNAs can be monitored in a stepwise fashion to form architectures of increasing complexity [9••], as there is a clear distinction between the energies involved in the formation of their 2°, 3° and 4° structures. In contrast to most DNA tiles, the formation of RNA tiles relies on the self-folding of single-stranded tectoRNAs that are characterized by well-defined 2° and/or 3° structures, and 4° intermolecular interfaces.

Nanoparticles, filaments and 2D RNA architectures

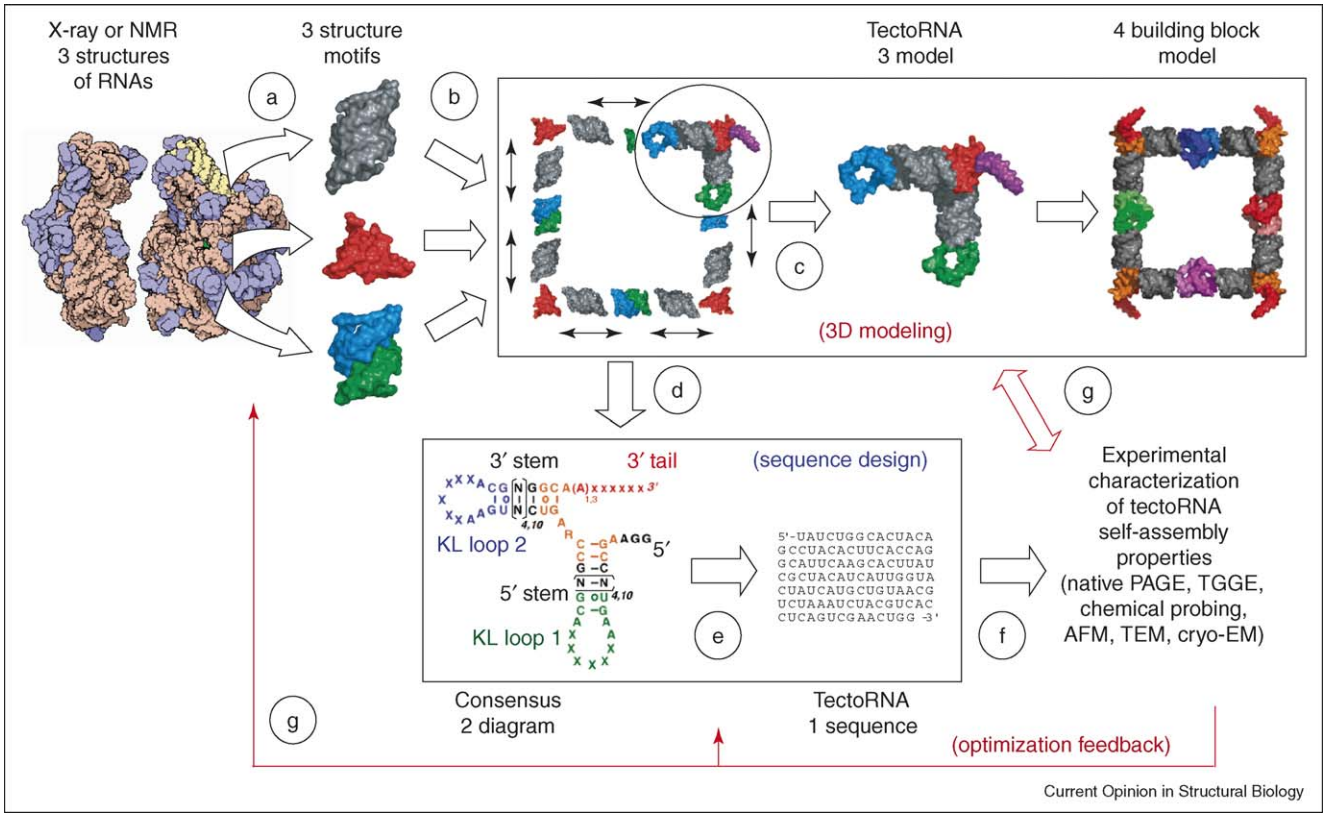
The first tectoRNAs to be generated by RNA architectonics self-assemble through loop–receptor interfaces to form dimeric nanoparticles [7,8,49] and micrometer-long RNA filaments [7,12•] (Figures 2 and 4). The atomic structure of a self-dimerizing loop–receptor tectoRNA particle was recently solved by NMR and shown to be in remarkable agreement with the initial 3° structure model [51•] (Figure 4a).

Combining rational design of well-defined RNA 3° structures with small-scale combinatorial synthesis offers great promise for engineering new functional modules that can accommodate the 3D constraints of specific supramolecular architectures [52–54]. For example, a new class of self-folding RNA molecule similar to domain P4-P6 of the natural *Tetrahymena* group I ribozyme (see glossary) was obtained by RNA architectonics [48] and used subsequently as a scaffold for combinatorial synthesis of new catalytic modules [54].

Several programmable and addressable (see glossary) RNA nanoparticles have been engineered to assemble

(Figure 2 Legend) Structural principles used in the nanoconstruction of RNA and DNA supramolecular building blocks. (a,b) Various examples of 2°, 3° and 4° structure motifs commonly used in (a) DNA and (b) RNA nanostructures. **(a)** As contiguous helices tend to stack on top of each other, it is possible to constrain the geometry of DNA helical elements to form three- or four-way junctions. One of the helices in the bulged three-way junction is generally more flexible than the two others [2]. The quintessential DNA 3° structure motif is the four-way (Holliday) junction [2]. For supramolecular 4° assembly, DNA units can be joined through non-covalent sticky tail connectors [32] (left) or through internal loop–loop interactions that fold into paranemic crossover junctions (right) that do not interpenetrate topologically [44]. **(b)** RNA 2° structure elements can form flexible hinges at the level of single-stranded regions, internal loops or multiway junctions. Specific sets of nucleotides can also direct the formation of a distinct helical geometry that leads to stable rigid 3° structure motifs [14,15] (from left to right): the right-angle motif [9••]; the internal loop E and kink-turn motifs [14,15]; the UA-handle and the A-minor loop three-way junction motifs (L Jaeger *et al.*, unpublished), two distinct three-way junctions that specify different helical geometries; the four-way junction motif from the hairpin ribozyme [7,12•]; and the class 2 tRNA five-helix junction motif. RNA 4° interactions used to generate supramolecular assemblies (from left to right): tail connectors [9••]; loop–loop ('kissing') interactions [9••,50]; and the double GNRA loop–receptor interaction [8]. **(c)** The principle of tensegrity for constructing rigid triangles [27], tetrahedrons [43•] and octahedrons [42••]. Tensegrity involves rigid struts (helices) that push outward and flexible tendons (junctions) that pull inward, creating stable rigid structures. Consequently, this strategy does not require rigid 3° structure motifs. **(d)** Examples of self-assembling DNA tiles (the number of constitutive molecules is indicated in parentheses). **(i)** Rhombus (four strands) [26]; **(ii)** single crossover triangle (four strands) [27,28•]; **(iii)** 4 × 4 cross (nine strands) [63]; **(iv)** three-point star (seven strands) [29–31]. (v–ix) Double-crossover (DX) tiles: **(v)** DAE (five strands); **(vi)** DAO (four strands); **(vii)** DAE with protruding helix (four strands) [32]; **(viii)** DX tile with protruding triangle (four strands) [33]; **(ix)** DX triangle (ten strands) [34]. (x–o) Helix-bundle (HB) tiles: **(x)** triple-double crossover tile (TDX) (four strands) [35]; **(xi,xii)** 4HB tile (eight or nine strands) [36,37•]; **(xiii)** 8HB tile (18 strands) [37•,38]; **(xiv)** 3HB tile (nine strands) [39]; **(xv)** 6HB tile (16 strands) [40]. **(e)** Architectures of RNA supramolecular building blocks. Basic molecular RNA units, called tectoRNAs, are assembled through 4° interactions to form self-assembling nanoparticles and filaments: **(i,ii)** loop–receptor tectoRNA dimeric particle [7,8,49]; **(iii,iv)** H-shaped tectoRNA particle and filament [7,12•]; **(v,vi)** small and large right-angle tectosquares [9••]; **(vii)** 'kink-turn' tectoRNA nanoparticle; **(viii,ix)** pRNA dimeric and trimeric particles [10,55]; **(x)** 'A-minor 3WJ' tectoRNA filament (C Geary, L Jaeger, unpublished); **(xi)** tRNA tectosquare (I Severcan *et al.*, unpublished).

Figure 3



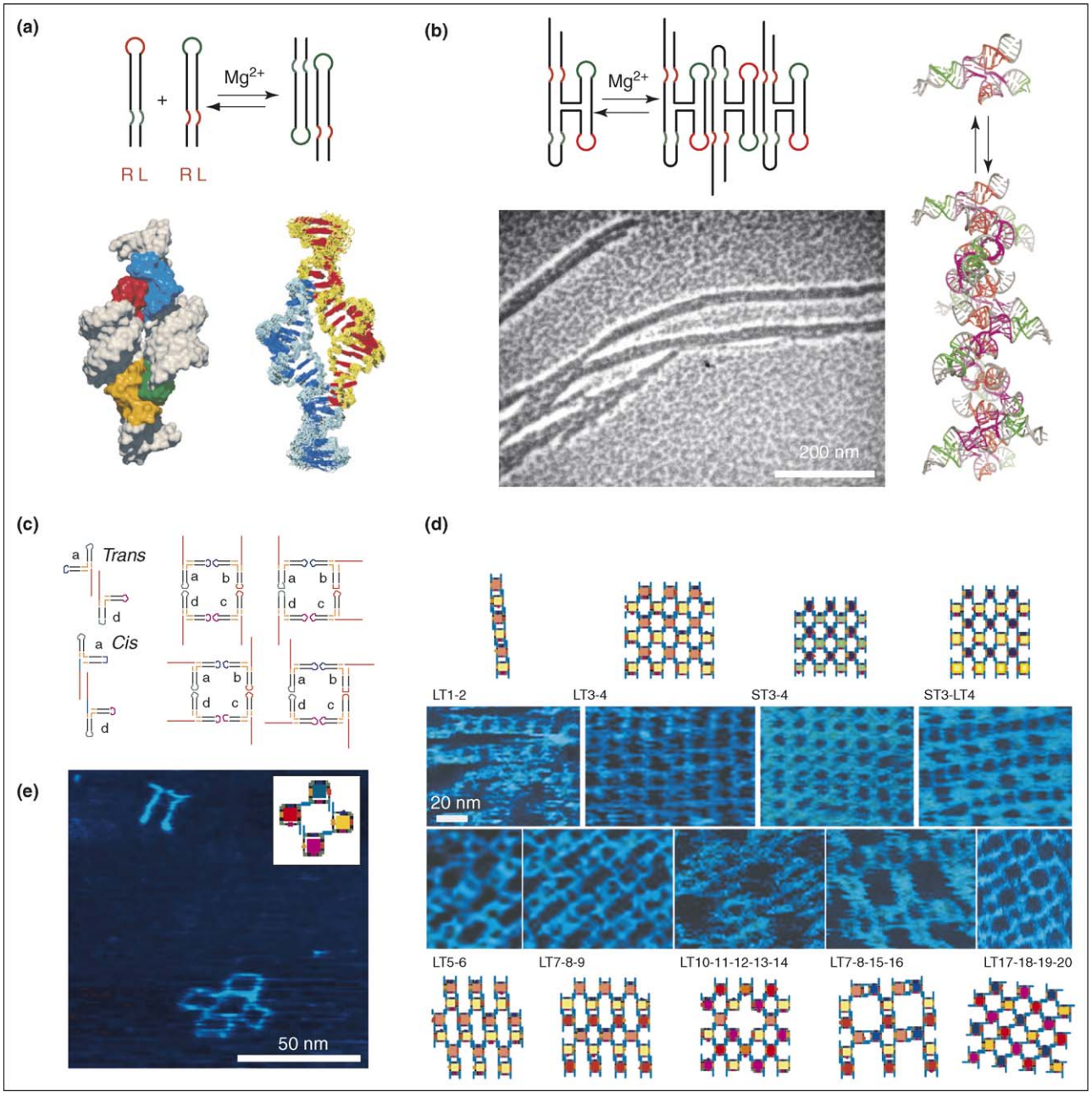
The RNA tectonics methodology. This multistep approach is both theoretical and experimental. The rational design of artificial 3D RNA architectures is based on an inverse folding process [7,8,9••,12•]. **(a)** Structural fragments corresponding to 3° structure motifs are 'cut and pasted' from known X-ray or NMR structures, and **(b)** interactively reassembled into novel tectoRNA architectures by computer modeling with graphic user interfaces. During this mosaic modeling process [18], 3° interacting motifs can be positioned and oriented precisely by adjusting the lengths of their linking helical elements and the stacking of the helices at multihelix junctions, thus allowing one to control the supramolecular assembly of RNA units. **(c)** TectoRNAs are predicted to assemble into supramolecular architectures based on the conformation and geometry of their constitutive structural elements. **(d)** These 3° models are then used as scaffolding to define consensus 2° diagrams, specifying invariant nucleotide positions to retain 3° structure constraints and positions involved in base pairing. **(e)** TectoRNA sequences able to fold into these 2° blueprints are optimized by energy minimization [23] to maximize their thermodynamic stability and minimize the occurrence of alternative 2° structure folds [24]. **(f)** The RNA sequences are synthesized by chemical or enzymatic methods (e.g. [8,9••]), and their expected folding and self-assembly properties characterized [9••,12•,47]. **(g)** The experimental data are then compared to the theoretical models and used to optimize the tectoRNA rational design at the sequence or 3D model level.

in a predictable fashion through complementary selective loop–loop interactions [9••,10,50,55]. The DNA-packaging motor of bacterial virus Φ29 contains six DNA-packaging RNAs (pRNAs), which together form a hexameric ring via loop–loop interactions. For example, pRNAs were redesigned to form a variety of predictable structures, namely dimers, tetramers, triangles, rods and micrometer-size bundles of pRNA filaments [10,55]. Recently, controllable trimeric pRNA particles harboring therapeutic molecules, siRNAs (see glossary) and a receptor-binding aptamer were demonstrated to act as a delivery vehicle to cancer cells and to induce apoptosis [11•].

Collinear kissing loop interactions can generate strong 4° intermolecular interfaces to promote the formation of RNA particles of different sizes [50] (Figure 2b). This assembly principle was used in the engineering of a versatile molecular system that takes advantage of a 'right-angle' 3° structure motif to form highly programmable square-shaped tetrameric nanoparticles, called tectosquares [9••] (Figures 3 and 4c).

The high modularity and hierarchical supramolecular structure of tectosquares makes it possible to construct a large number of them from a limited set of tectoRNAs that assemble through strong 4° interaction loop–loop interfaces [9••]. Mixtures of tectosquares that display a variety of sticky tail connectors at their corners to control the geometry, directionality and addressability of self-assembly can assemble further into complex 1D and 2D architectures with periodic and aperiodic patterns and finite dimensions (Figure 4c–e). Considering that up to

Figure 4



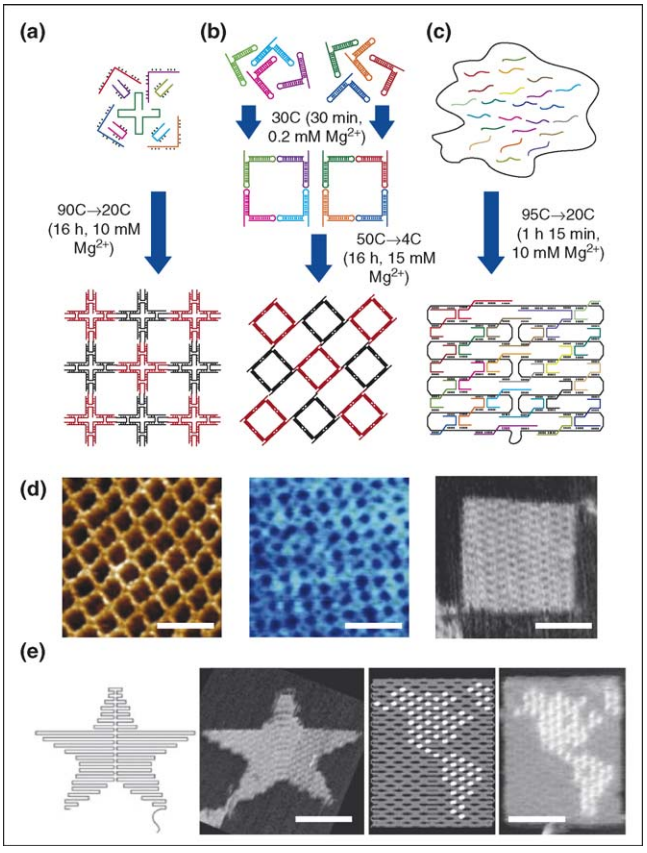
Programmable supramolecular RNA architectures. **(a)** 0D loop–receptor (RL) dimeric tectoRNA particle: the original 3° structure model (left) [7,8] is in remarkable agreement with the recently determined NMR structure of the particle (right) [51•]. **(b)** As predicted by 3° structure models (right), H-shaped tectoRNAs can assemble into programmable, chiral and directional RNA filaments (1D) that can be visualized by TEM (adapted from [12•]). **(c)** RNA tectosquares are programmable tetrameric nanoparticles. The geometry of tectosquare assembly can be controlled by the orientation and length of their 3′ tail connectors [9••]. (d,e) 2D architecture of tectosquares (adapted from [9••]). **(d)** Various periodic patterns generated by various combinations of 22 tectosquares. **(e)** The first programmable RNA nanogrid, with 16 distinct addressable positions [9••]. This RNA structure is aperiodic with respect to its molecular constituents.

88.5 million distinct tectosquares can be theoretically synthesized from a limited set of 24 tails with two different tail orientations and sizes, an almost infinite number of complex jigsaw puzzle patterns can be designed [9••].

Strategies for programmable nucleic acid self-assembly

Two main approaches can be distinguished for programmable self-assembly of nucleic acid architectures (Figure 5). The first approach, mostly used with

Figure 5



The main strategies for programmable self-assembly. **(a)** Single-step self-assembly: all the molecules are mixed together and assembled through a slow cool annealing procedure (most DNA architectures are formed this way). **(b)** Stepwise hierarchical self-assembly [9••,58]: specific sets of molecules are first separately assembled into small supramolecular entities that are then mixed in a stepwise fashion to form the final architecture. Hierarchical assembly is favored by the use of 4° interactions with different stabilities and magnesium requirements. **(c)** Scaffolded self-assembly or scaffolded DNA origami: a long single-stranded molecule is folded into an arbitrary shape with small oligonucleotides acting as staples [46••]. A variation of this methodology is the directed nucleation self-assembly strategy, in which DNA acts as a template for the subsequent assembly of oligonucleotide tiles [62]. **(d)** From left to right: AFM images of a 4 × 4 cross array (adapted with permission from [63]), a tectosquare array [9••] and a DNA origami array (adapted with permission from [46••]), assembled through the methods described in (a–c), respectively. Taking into account the way molecules can be functionalized, the 4 × 4 cross array, the RNA array and the DNA origami array have pixels of 14 nm, 5 nm and 3.6 nm, respectively. The scale bar is 50 nm. **(e)** Two examples of scaffolded DNA origami: folding of the M13mp18 DNA (7176 nucleotides) into a five-point star shape with ~200 oligostaples; and drawing of a map of the Americas by patterning a DNA nano-board of 217 oligopixels (adapted with permission from [46••]). The scale bar is 50 nm.

DNA, is a single-step assembly strategy in which all the molecules encoding a specific architecture are mixed together and assembled in 'one pot' through a slow annealing procedure (e.g. [26,27,28•,29–36,37•,38–40,46••,56]) (Figure 5a). According to the energetics of their 2° structure pairings, oligonucleotide strands form stable substructures or tiles that assemble through weaker 4° interactions into larger nanoarchitectures when lower temperatures are reached. These structures can be ligated to form robust covalently linked architectures [57] or networks [32].

The second approach, particularly appropriate for RNA assembly, is a stepwise hierarchical self-assembly strategy, in which various small subunits are first separately formed and then mixed together to form the final supramolecular architecture (Figure 5b) [9••,58,59]. This strategy is more time consuming. However, as exemplified by the tectosquare system [9••], it can make use of the same 4° interactions and basic molecular units to build a large number of highly modular tiles that can assemble further through weaker 4° interactions. Thus, by separating tile formation from the formation of larger supramolecular assemblies, a reduced number of different connecting interfaces can be used to hierarchically build highly modular architectures [9••]. In stepwise assembly, the melting temperature of the tiles and of the resulting supramolecular architecture should be kept well separated. By contrast, this is not absolutely necessary for the one-pot approach, as exemplified by scaffolded DNA origami [46••].

Stepwise assembly can be used to generate programmable architectures of finite size, with the position of each of the constitutive molecules known and therefore addressable within the final architecture. The first demonstration of this approach led to the fabrication of RNA nanogrids of finite size (Figure 4e) [9••,59]. More recently, the application of this strategy to DNA led to the fabrication of nanogrids with precisely positioned nanoparticles that form patterns of letters [58] or a pegboard [60].

Each of these approaches can make use of additional non-mutually exclusive self-assembly strategies, such as algorithmic self-assembly, directed nucleation (or templated) self-assembly and scaffolded self-assembly. In algorithmic self-assembly, a set of nucleic acid tiles, defined as Wang tiles (see glossary), is viewed as the algorithm for a particular computational task leading to the formation of 1D, 2D and 3D patterns. This strategy was used to compute the formation of aperiodic fractal 2D patterns based on the Sierpinski triangle pattern [61•]. To achieve this task, a minimal set of four DNA tiles with local pairing rules designed to implement the exclusive-or (XOR) function was assembled on a template input row to facilitate the nucleation of directional self-assembly into a unique pattern [61•]. The potential of algorithmic self-assembly is, however, still limited by the presence of various errors, introduced by lattice dislocation, formation of untemplated crystals and mismatched tiles.

Templated or directed nucleation assembly takes advantage of a nucleic acid template that acts as a scaffold for directing the specific assembly of tiles. This strategy led to the formation of aperiodic 2D arrays, such as DNA barcodes [62]. The construction of a replicable DNA octahedron [42••] was based on a similar scaffolded approach. In this case, a single-stranded DNA molecule that forms helical struts was assembled with the help of four small oligonucleotides into its final shape through the formation of paranemic long-range interactions (Figure 2a). The generalization of these approaches led to the versatile scaffolded self-assembly strategy, also called scaffolded DNA origami [46••], which can generate with remarkable efficiency any arbitrary shape and pattern (Figure 5c,e). In this strategy, a long single-stranded DNA scaffold is folded with complementary oligonucleotides that act as staples. The desired shape is designed by raster-filling the shape with a 7 kilobase single-stranded scaffold and ~200 short oligonucleotide staple strands to hold the scaffold in place. Once synthesized and mixed, the staple and scaffold strands self-assemble in one single step. The structure can be programmed into complex patterns, such as words and images (Figure 5e). The success of scaffolded DNA origami stems from several contributing factors, such as efficient strand invasion, excess of staples, cooperative effects and a design that intentionally does not rely on binding between staples. A relatively good yield of defect-free DNA architectures was obtained, despite the fact that the oligonucleotides used were not purified.

Additional principles of nucleic acid architectonics

Principle of orientational compensation

The inherent asymmetric nature of RNA and DNA tiles can have a dramatic effect on the larger nanostructures that they form by introducing various degrees of curvature. By using the principle of orientational compensation, whereby two adjacent units are related by a local twofold pseudo-rotational axis of symmetry, one source of asymmetry can be locally eliminated, so that asymmetric tiles that are not perfectly flat can still assemble in a plane instead of forming nanotubes [30,33,63]. This strategy was also used to favor the assembly of 'H-shaped' tectoRNAs into linear filaments instead rings [12•] (Figure 4Figure 4b).

Application of principles of symmetry

The application of sequence symmetry principles to the design of structurally symmetrical tiles can reduce the sequence size and number of strands necessary for the construction of very complex nanostructures. This approach was extremely powerful for fabricating 2D DNA arrays up to 1 mm in size [64•]. Similarly, the application of symmetry principles to tile assembly can reduce dramatically the number of tiles when constructing nanoarrays of finite size [9••,38].

Fractal nanoarchitectures

Fractal patterns can be generated by algorithmic self-assembly [61•]. The use of hierarchical stepwise assembly strategies to build fractal architectures remains to be demonstrated [65].

Ornamentation of nucleic acid architectures

Principles for the ornamentation of DNA architectures have been reviewed elsewhere [4•]. Briefly, programmable nucleic acid architectures can direct the spatial organization of other components, such as proteins [28•,63,66], metallic nanoparticles (e.g. [58,60,67,68]), small molecules and nanodevices [3], generating new materials with potential applications in fields as diverse as medicine, molecular biology and device physics [4•]. Among the various strategies employed for functionalizing nucleic acid architectures, the use of DNAzymes, ribozymes, therapeutic siRNAs, and RNA and DNA aptamers is particularly promising, as these molecules can be readily encoded at precise locations within the nucleic acid architecture (e.g. [11•,66]).

DNA self-assembling 1D architectures can serve as a template for the fabrication of highly conductive silver nanowires by electroless chemical deposition techniques (e.g. [39,63]). Recently, conductive self-assembling nanowires were constructed by assembling gold-derivatized DNA particles with loop–receptor tectoRNAs [69]. Another interesting feature offered by well-defined nucleic acid architectures such as 1D tectosquare ladders (Figure 1) is their use as scaffolds for controlling the positioning of cationic nanoparticles based on electrostatics, size and shape recognition [70].

Conclusions

Is the dream of achieving the total control of matter at a molecular level close to becoming true? What is meant by 'total control' is a highly debatable question, as nothing in this universe will ever be totally under human control. Nevertheless, the recent scientific breakthroughs presented herein lead us to believe that exquisite control over the shape, growth, movement, recognition and catalytic behavior of molecular architectures will be achieved for nucleic acids in the future [2].

The great potential of DNA architectonics is best exemplified by the quasi-digital approach of scaffolded DNA origami. DNA can be shaped into arbitrary 1D, 2D and 3D architectures with sizes ranging from 20 nm to 200 nm or more, and any type of pattern can be drawn with DNA with a pixel definition of 3.6 nm [46••]. By contrast, the great potential of RNA architectonics lies in the possibility of sculpting arbitrary shapes with sizes ranging from 1 to 25 nm, and with moving parts that can be precisely coordinated to generate responsive and directed molecular motion. At the present time, this potential is best

exemplified by complex natural RNA nanoparticles such as the ribosome [13].

Several challenges remain to be overcome, however. The efficiency of formation of nucleic acid nanostructures would be improved by minimizing errors that occur during folding and supramolecular assembly. The development of computer tools for facilitating the design and prediction of complex 3D nucleic acid structures, such as a compiler for the 3D structure language of nucleic acids, would be particularly helpful for achieving this task, especially in the case of RNA. It will also be important to explore further the principles of nucleic acid architectonics to achieve better control over the movement, dynamics and responsiveness of nucleic-acid-based nanomachines. For instance, DNA-based nanomechanical devices [3] are still far from matching the remarkable complexity and efficiency of RNA nanomachines such as the ribosome [13].

Because of the biodegradability and biological functions of RNA, programmable RNA architectures might be well suited to bionanotechnology and nanomedicine applications, whereas the robustness and chemical stability of DNA might offer greater possibilities for more conventional nanotechnology applications. In the near future, however, it is likely that the complementary nature of RNA and DNA will also find interesting new developments once mixed together!

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