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Computational Approaches to Nucleic Acid Origami

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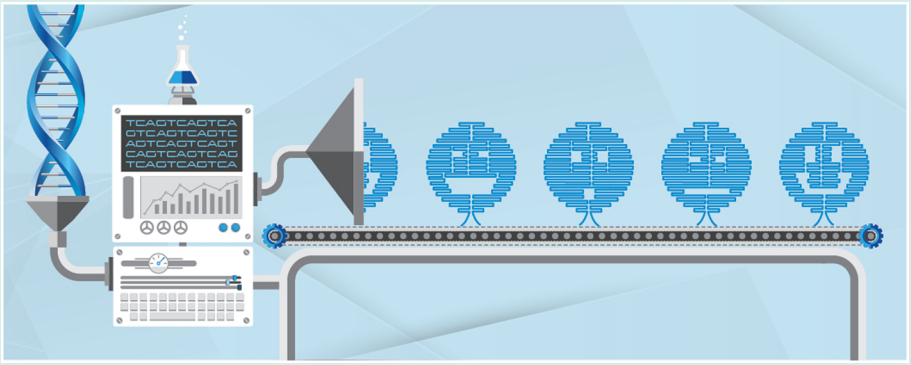

ABSTRACT: Recent advances in experimental DNA origami have dramatically expanded the horizon of DNA nanotechnology. Complex 3D suprastructures have been designed and developed using DNA origami with applications in biomaterial science, nanomedicine, nanorobotics, and molecular computation. Ribonucleic acid (RNA) origami has recently been realized as a new approach. Similar to DNA, RNA molecules can be designed to form complex 3D structures through complementary base pairings. RNA origami structures are, however, more compact and more thermodynamically stable due to RNA's non-canonical base pairing and tertiary interactions. With all these advantages, the development of RNA origami lags behind DNA origami by a large gap. Furthermore, although computational methods have proven to be effective in designing DNA and RNA origami structures and in their evaluation, advances in computational nucleic acid origami is even more limited. In this paper, we review major milestones in experimental and computational DNA and RNA origami and present current challenges in these fields. We believe collaboration between experimental nanotechnologists and computer scientists are critical for advancing these new research paradigms.

KEYWORDS: *DNA origami, RNA origami, computational approach, nanotechnology*

INTRODUCTION

In 1982, the field of DNA nanotechnology began with Nadrian Seeman's dream of organizing proteins in 3D crystals to study their structure with X-ray crystallography.[1] Since then, DNA molecules have been considered the building blocks of many nanostructures, and structural DNA nanotechnology was born. Through hybridization of complementary base pairs, DNA molecules provide the ability to construct nanoscale materials with control over the placement of each component tailored to a specific application. Within the last three decades, the field of DNA nanotechnology has delivered various advances in the control of matter on the nanoscale. In 1991, Chen and Seeman[2] presented the first DNA cube (Figure 1(a)), as an example of a DNA object with complex connectivity. With the identification of DNA double-crossover molecules in 1993[3] (Figure 1(b)), Winfree et al. designed the first 2D DNA crystal that forms from DNA tiles in a self-assembly process in 1998[4] (Figure 1(c)). Shih et al.[5] folded a 1.7 kb single-stranded DNA scaffold into an octahedron in 2004, which was later called "DNA origami" (Figure 1(d)). In 2006, Paul Rothemund introduced a design procedure for DNA origami that used a long single-stranded DNA scaffold and many short single-stranded DNA staples to produce different shapes[6] (Figure 1(e)). We note that, using the scaffolded DNA origami approach, one needs to design the process specifically for a particular shape.

Because of emerging interest in DNA origami and an increasing level of technical capability, the first three-dimensional DNA structures were produced in 2009 using single-layer DNA origami[7−10] (Figure 1(f)), multilayer DNA origami[11,12] (Figure 1(g)), and exploring the curvature of the 3D structure[13] (Figure 1(h)). Woo and Rothemund showed in 2011 that geometric arrangement of blunt-end stacking interactions in DNA can be used to create non-base pairing structures[14] (Figure 1(i)). In the same year, Han et al. generated complex hollow three-dimensional DNA origami structures[15] (Figure 1(j)). In 2012, Wei and colleagues introduced a cost-effective way to generate arbitrary DNA shapes using one set of short single-stranded DNA tiles[16] (Figure 1(k)). This idea was then generalized to the fabrication of distinct three-dimensional shapes[17] (Figure 1(l)). In 2014, Shi et al. reported a new DNA

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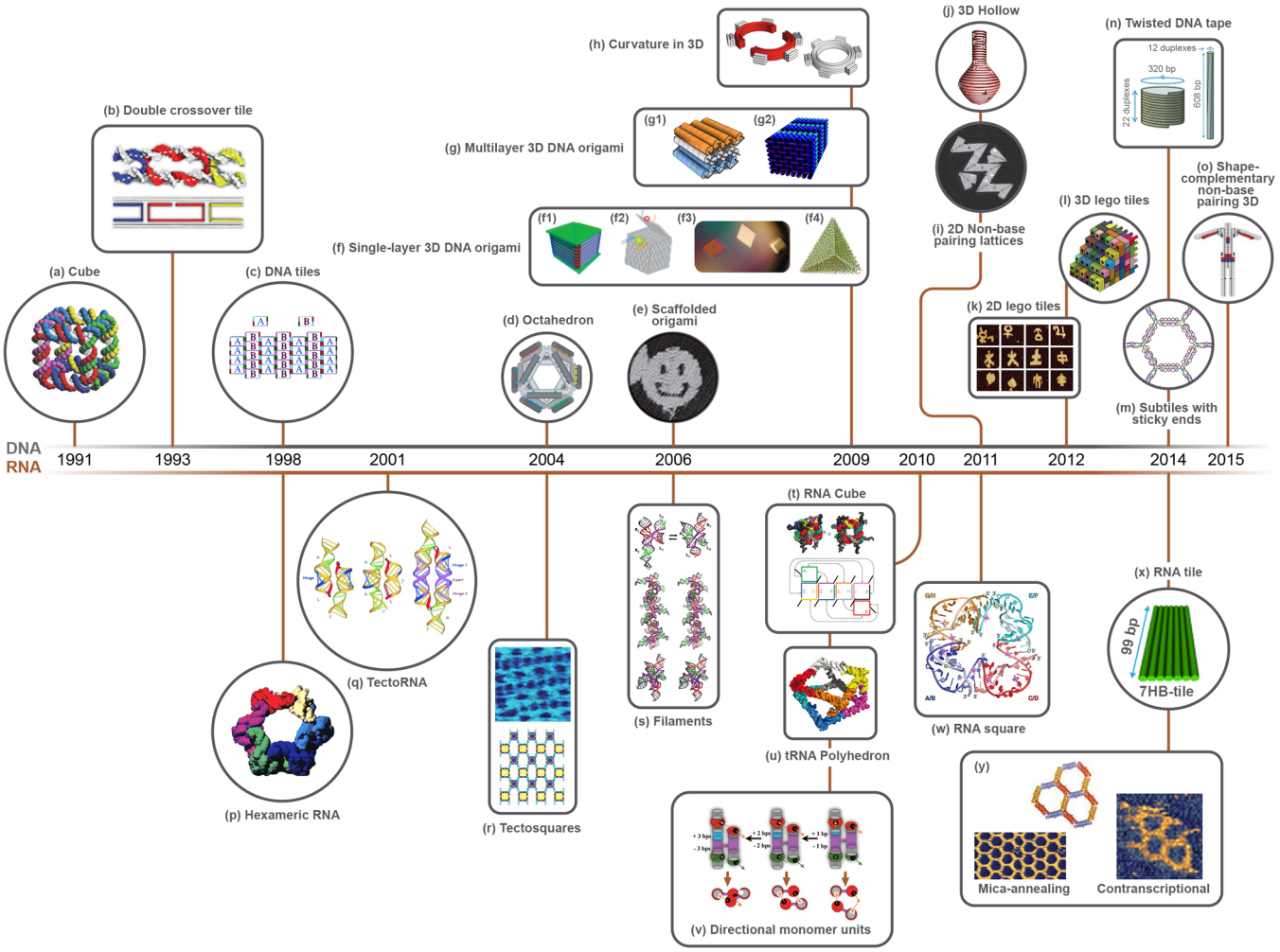

Figure 1. Important milestones in experimental DNA (above the horizontal line) and RNA origami (below the horizontal line). (a) cube, (b) double crossover tile, (c) DNA tiles, (d) octahedron, (e) scaffolded origami, (f) single-layer 3D DNA origami, (g) multilayer 3D DNA origami, (h) curvature in 3D, (i) 2D non-base pairing lattices, (j) 3D hollow, (k) 2D lego tiles, (l) 3D lego tiles, (m) subtiles with sticky ends, (n) twisted DNA tape, (o) 3D shape-complementary non-base pairing, (p) hexameric RNA, (q) Tecto-RNA, (r) Tecto-squares, (s) RNA filaments, (t) RNA cube, (u) tRNA polyhedron, (v) directional monomer units, (w) RNA square, (x) RNA tile, (y) contrascriptional origami. Images reproduced with permission from (a) ref 21, 2014, American Chemical Society (ACS); (b) ref 22, 2011, Nature Publishing Group (NPG); (c) ref 4, 1998, NPG; (d) ref 5, 2004, NPG; (e) ref 6, 2006, NPG; (f1) ref 7, 2009, Royal Society of Chemistry (RSC); (f2) ref 8, 2009, NPG; (f3) ref 9, 2009, NPG; (f4) ref 10, 2009, ACS; (g1) ref 11, 2009, NPG; (g2) ref 12, 2009, ACS; (h) ref 13, 2009, The American Association for the Advancement of Science (AAAS); (i) ref 14, 2011, NPG; (j) ref 15, 2011, AAAS; (k) ref 16, 2012, NPG; (l) ref 17, 2012, AAAS; (m) adopted and redrawn from ref 18; (n) ref 19, 2014, Wiley; (o) ref 20, 2015, AAAS; (p) ref 23, 1998, Cell Press; (q) ref 24, 2001, Oxford University Press (OUP); (r) ref 25, 2004, AAAS; (s) ref 26, 2006, OUP; (t) ref 29, NPG; (u) ref 28, 2010, NPG; (v) ref 27, 2010, OUP; (w) ref 30, 2011, Proceedings of the National Academy of Sciences (PNAS); (x) ref 32, 2014, Wiley; and (y) ref 31, 2014, AAAS.

subtile strategy for the design of two-dimensional DNA structures[18] (Figure 1(m)). DNA subtiles consist of three single-stranded DNA molecules with two levels of sticky ends and a hierarchical self-assembly process. Endo et al.[19] presented a new design method to create helical tubular structures that, instead of connecting both edges of DNA tiles along the helical axes, uses winding DNA tape to form a helical assembly (Figure 1(n)). Gerling et al. demonstrated in 2015 that three-dimensional DNA shapes can self-assemble without base pairing on the basis of geometric arrangement or shape-complementarity[20] (Figure 1(o)).

Many applications have emerged from DNA origami objects[21,33,34] because of its unique properties, such as an addressable surface, and versatility. The majority of applications are focused on DNA origami objects with static geometries, such as a template to organize proteins[35] and nanoparticles,[36,37] vesicles for drug delivery,[10,38,39] nanocapsules,[40] imaging of molecular motion,[41,42] biosensors,[43−45] electronic logic gates,[46] and nanopores;[47−49] other studies make a step forward in the design of dynamic DNA devices (i.e., DNA nanomachines), such as DNA tweezers,[50] walkers through prescribed tracks, landscapes,[51−53] and transformers[54] (Figure 2).

Similar to DNA, RNA is a polymer of four nucleotides that forms base pairs through hybridization and thus provides the ability to construct nanoscale materials. Despite their resemblance, there are differences between DNA and RNA molecules. RNA is a single-stranded molecule transcribed from DNA that folds on itself to form stems and loops. RNA nucleotides are adenine (A), cytosine (C), guanine (G), and uracil (U), whereas DNA contains thymine (T) instead of uracil. Although base pairing is through complementary (canonical) hybridization in DNA molecules (i.e., A pairs

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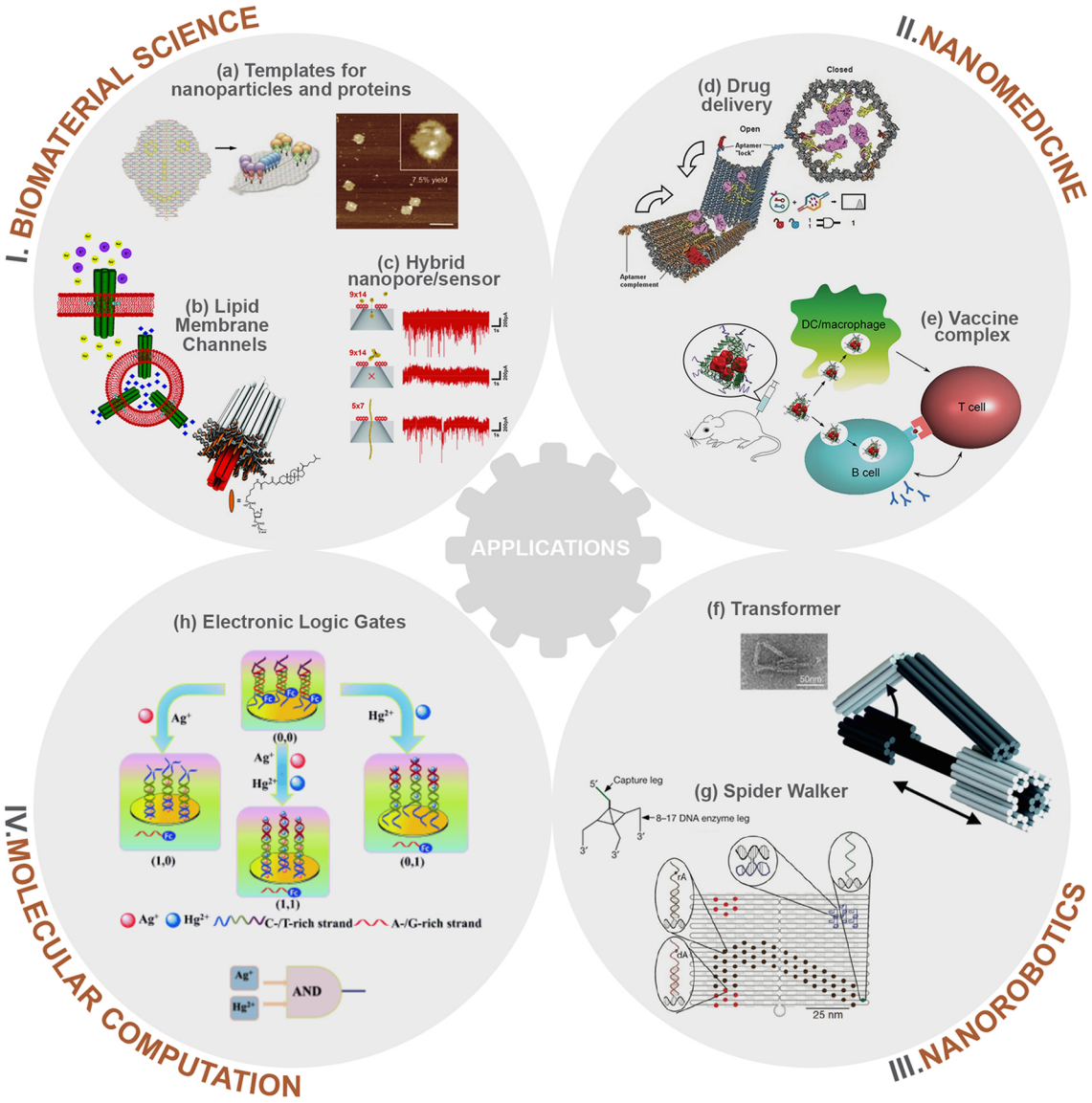

Figure 2. Examples of applications of DNA origami in different fields. (I) Biomaterial science: (a) Schematic (left) and AFM (right) templates for nanoparticles and proteins: site-specific decoration of DNA origami with self-labeling fusion proteins. (b) Schematic representation of the side and top view (left, middle) of the DNA transmembrane channels in lipid bilayers. The DNA transmembrane channel (right) is composed of a stem that can penetrate to a lipid membrane and a barrel-shaped cap decorated with cholesterol moieties that adhere to the membrane. (c) Hybrid nanopore/sensor: schematics and current−time traces of translocations of streptavidin (top), the absence of translocations of immunoglobulin G (middle), and the presence of a translocation signal for double helical linear DNA (bottom) through a DNA origami nanoplate with a (9 nm × 4 nm) pore trapped in a SiN solid state nanopore. (II) Nanomedicine: (d) Barrel-like aptamer-gated nanorobot programmed to be open in the presence of the specific target cells using partially complementary strands as keys and to deliver payloads, such as gold nanoparticles or Fab fragments. (e) Schematic design of a DNA scaffolded adjuvant-antigen vaccine complex. CpG ODN adjuvant molecules (ribbons attached to DNA tetrahedron) and the model streptavidin antigen (red balls) bind specifically to B cells and are subsequently presented to T cells to activate B cell response and antibody production. (III) Nanorobotics: (f) DNA origami crank slider machine that couples linear and rotational motion. (g) DNA spider with three legs consisting of a DNAzyme programmed to start, walk, and stop into the predesigned track by binding and cleaving RNA (rA)-containing DNA strand introduced on the DNA origami substrate. (IV) Molecular computation: (h) Metal-ion-mediated DNA logic gates (using AND, NAND, and NOR) with Ag^{2+} and Hg^{2+} ions as input and electrochemical signals as output. (a) ref 35, 2010, Wiley; (b) ref 55, 2014, RCS and ref 48, 2014, AAAS; (c) ref 48, 2012, AAAS; (d) ref 38, 2012, AAAS; (e) ref 56, 2012, ACS; (f) ref 57, 2015, National Academy of Sciences; (g) ref 51, 2010, NPG; and (h) ref 46, 2013, Wiley.

with T and C pairs with G), in RNA molecules, non-canonical base pairings can form in addition to the complementary hybridization (i.e., similar to DNA, A pairs with U and C with G, although G may also pair with A or U). This difference in base pairing allows RNA molecules to form distinct motifs from those of DNA. RNA molecules are also found to be more thermodynamically stable than DNA molecules.[58] They play various functional roles in a cell, ranging from translation and regulation of genes to coding for proteins,[59−64] and their functions are mainly determined by their complex three-dimensional structures.[65−68] RNA folding is hierarchical,[69−72] and RNA tertiary structure is mainly determined by its secondary structure[69] (i.e., set of base pairs). Because of their central role in complex biological machinery, the programm-

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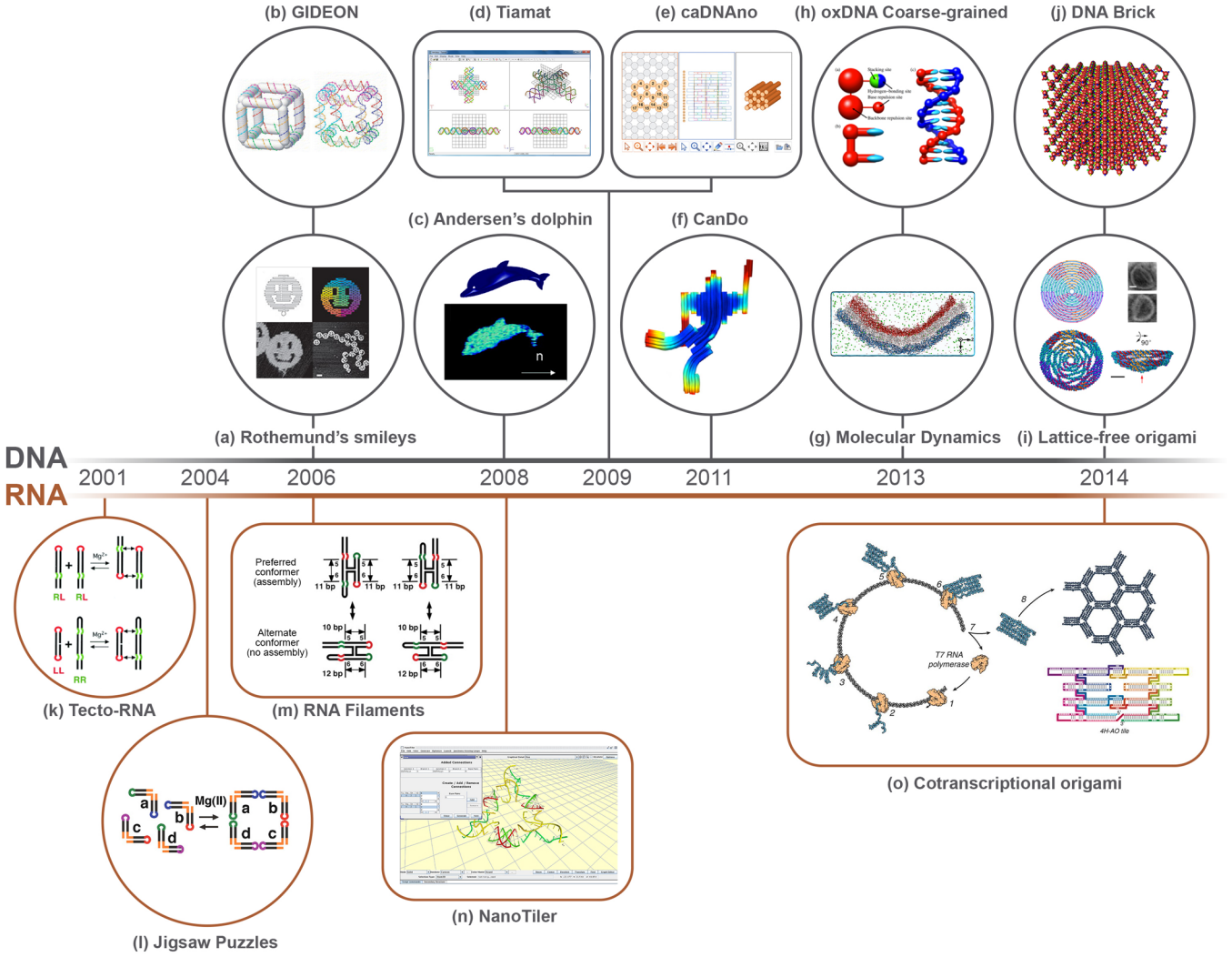

Figure 3. Important milestones in computational DNA (above the horizontal line) and RNA origami (below the horizontal line). (a) Rothemund's smileys, (b) GIDEON, (c) Anderson's dolphin, (d) Tiamat, (e) caDNAano, (f) CanDo, (g) molecular dynamics, (h) oxDNA coarse-grained, (i) lattice-free origami, (j) DNA brick, (k) Tecto-RNA, (l) jigsaw puzzles, (m) RNA filaments, (n) Nanotiler, and (o) cotranscriptional origami. Images reproduced with permission: (a) ref 6, 2006, NPG; (b) ref 84, 2006, Elsevier; (c) ref 85, 2008, ACS; (e) ref 88, OUP; (f) ref 22, 2011, NPG; (g) ref 90, 2013, PNAS; (h) ref 91, 2013, RSC; (i) ref 89, 2014, NPG; (j) ref 92, 2014, ACS; (k) ref 24, 2001, OUP; (l) ref 25, 2004, AAAS; (m) ref 26, 2006, OUP; (n) ref 93, 2008, Elsevier; and (o) ref 31, 2014, AAAS.

ability of their secondary structures, and their hierarchical folding, RNA molecules are well positioned to be designed to perform complex dynamic functions in nanotechnology.

Despite the similarities between DNA and RNA molecules in providing the necessary requirements for the construction of nanoscale structures, RNA nanotechnology was not realized until the late 1990s when Guo and his team demonstrated the construction of RNA nanoparticles using re-engineered pRNA to self-assemble by hand-in-hand interactions into multimeric RNA nanoparticles[23] (Figure 1(p)). In 2001, Jaeger et al. introduced "Tecto-RNA" molecular units constructed based on non-base pairing tertiary interactions in RNA molecules that are capable of self-assembly to generate a wide range of nanoscale structures[24] (Figure 1(q)). These tertiary interactions, called loop-receptor interactions, are based on interaction between a GAAA loop and a GAAA loop receptor (shown in red and green respectively in Figure 3(j)). Each Tecto-RNA can interact with two other Tecto-RNAs through two such tertiary interactions. In 2004, Chworos and colleagues designed "Tecto-squares", three-dimensional RNA building blocks capable of algorithmic self-assembly into different complex nanostructures[25] (Figure 1(r)). They showed that the stability and rigidity of Tecto-squares make these modular units particularly attractive for the construction of programmable planar supra-architectures.[25] In 2006, Nasalean et al. demonstrated directional self-assembly of Tecto-RNA units to form filaments[26] (Figure 1(s)). To control the assembly direction, they found that the position of the four way junction (4WJ), where four helices meet, has to be adjusted so that the distance separating the modules is only 11 base pairs in the desired conformation, and they incorporated an additional loop/loop-receptor pair to Tecto-RNA's design to facilitate this. It was in 2010 when Novikova et al. achieved tuning of the stoichiometrics of self-assembled complexes by carefully positioning interaction motifs in monomer units[27] (Figure 1(t)). They systematically studied different configurations and introduced a molecule with desired properties (molecule 8). Also in 2010, Severcan et al.[28] used tRNA structures as building blocks for creating a stable 3D polyhedron, which highlights the controllability of RNA structural units as building blocks of 3D

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structures (Figure 1(u)). In the same year, Afonin et al.[29] designed and developed RNA cubes of 6 to 10 strands with or without dangling ends using a one-pot self-assembly process (Figure 1(v)).

By designing a small, compact asymmetric RNA square from double stranded RNA molecules in 2011, Dibrov et al. provided a platform for directional sequence-dependent self-assembly of nano structures of up to four modular units[30] (Figure 1(w)). Using the scaffolded DNA origami technique, Endo et al.[32] created scaffolded RNA origami bricks and tubes with average yields of approximately 50 and 45%, respectively (Figure 1(x)).

Despite all efforts on RNA nanotechnology, structures made by RNA have been limited in size to a maximum of 200 nucleotides, whereas structures built from DNA can be up to 45000 nucleotides.[31] To alleviate this restriction, Geary et al. designed a scalable method using hairpins and kissing loops for the construction of RNA two-dimensional structures by either heat-annealing over mica or cotranscriptional folding[31] (Figure 1(y)). Cotranscriptional folding refers to the process of RNA folding that occurs during RNA transcription. Two underlying factors for cotrascriptional folding of RNA molecules are (1) a slower transcription rate compared to the rate of folding, and (2) favorable ionic conditions of the cellular environment. Geary et al. used these facts to assemble RNA tiles. They also introduced a new type of crossover pattern, called a "dovetail seam", for creating sequence-specific and non-topologically linked arrays of coaxially stacked helices.

As a natural biopolymer that provides necessary structural requirements for constructing nanostructures, RNA molecules have been used in similar applications as DNA molecules, including cellular delivery,[73] cancer therapy,[74] and molecular computation.[75] However, because of their higher stability at low PH, RNA molecules are viewed as better candidates for therapeutic applications than DNA molecules.[58] With the identification of RNA riboswitches, molecules that change their structures based on difference in the environment, RNA molecules have been used to perform logical operations.[76] Artificial ribozymes have been constructed from RNA molecules that perform novel catalytic activities.[77] For a comprehensive review of applications of RNA molecules in nanotechnology or in molecular computation, we refer interested readers to the work of Peixuan Guo[58] and Qui et al.,[75] respectively.

The first study of DNA−RNA structures dates back to late 1990s;[78] however, self-assembly of DNA−RNA hybrids for creating nanoscale structures did not happen until 2010 when Ko et al.[79] introduced a general design strategy for assembly of RNA strands programmed by DNA strands by utilizing complementary base pairing between DNA and RNA strands while ensuring that all helical domains were hetero-RNA−DNA duplexes. Their designed structures posed different symmetry that were more thermodynamically stable but less chemically stable compared to similar structures designed by homo-DNA strands. In 2013, Endo et al.[80] presented RNA-templated DNA origami structures using RNA transcripts and designed staple DNA strands. Their designed structures were smaller in size than their corresponding homo-DNA origami structures, suggesting that helices in RNA−DNA hybrid tiles pack more tightly than in homo-DNA tiles. Furthermore, by chemically modifying uracils (U) in RNA templates, the authors showed that the physical properties of assembled structures could be changed. Inspired by isothermal DNA assembly,[81] Wang et al.[82] reported a fast and robust general design strategy for generating DNA−RNA hybrid origami structures using an RNA strand as scaffold and DNA staples. They reported the formation of hybrid origami on the order of 5 min but with lower yield than possible for homo-DNA origami. They showed that the ratio of staple strands to scaffold is 1 to 1, whereas in DNA origami, an excess amount of staple strands are needed.

Hybrid origami structures have been recently used in designing nanowires[83] and in nanoparticles designed to conditionally activate RNA interference in various human cells.[73]

Computational approaches can be used to guide the design of new DNA and RNA structures and optimize sequence requirement for controlling the direction and geometry of nanostructures. In fact, in many instances, computational frameworks were used to test and or verify the results (see, for examples, refs 26, 27, and 31). However, as we will discuss later in this paper, the development of a computational framework for DNA and RNA origami lags behind their experimental development. Figure 3 summarizes the major computational milestones for DNA and RNA origami. The aim of this work is to provide a review of computational approaches to DNA and RNA origami and the challenges that need to be addressed to make a step forward in the realization of their applications.

In this paper, we first provide a brief description of computational milestones in the development of DNA and RNA origami. Then, we review some of the major developments in applying molecular dynamic and coarse-grained simulations to the field of DNA and RNA origami. Finally, we enumerate some of the current challenges of this research field and provide possible future research directions.

COMPUTATIONAL DNA ORIGAMI

As we mentioned previously, a methodological approach for scaffolded DNA origami was proposed in 2006 by Paul Rothemund, in which he explained the exact process of creating scaffolded DNA origami as follows[6] (Figure 3(a)). Rothemund explained his design process in five steps ((1) creating a geometric model of the DNA structure, (2) filling the shape by an even number of parallel double helices, (3) incorporating crossovers to keep the helices in place, (4) optimizing the crossovers, and finally, (5) finishing the design after merges and rearrangements along the seam), the first two of which were done manually. These five steps created a step-by-step procedure, or "algorithm", that results in the successful creation of the desired shape. This is why we consider this work as the first computational attempt at DNA origami, although Rothemund experimentally evaluated his algorithm by generating multiple nano-objects.

Shortly after publication of Rothemund's scaffolded origami, a new computational method named "GIDEON" was published by Birac et al.[84] (Figure 3(b)). GIDEON is a graphical integrated development environment that facilitates the development and evaluation of simple models of structural DNA nanotechnology. It encapsulates structural connectivity in the form of linked arrays. In this model, each nucleotide is represented as a circle connected to other nucleotides with lines. Each nucleotide keeps the following information: a reference to its complementary pair, its associated base type (i.e., A, C, G, or T), and a reference to its neighboring bases in the backbone. A "strand" data structure, then, is a linked list of nucleotides of the backbone, and a duplex data structure keeps information on the bases that pair and their central axis. To

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align the components of a structure to its correct geometric structure, GIDEON has a relaxation algorithm that gradually rearranges the structure to minimize mechanical, planar, and torsion strains. Users have the ability to add and remove nucleotides and change the position of duplexes. Using this geometric but non-energy-based model, GIDEON is useful in checking for steric clashes.

In 2008, Andersen et al.[85] presented a software package for designing DNA origami structures for SARSE, a semiautomated RNA sequence editor that combines tools for analyzing RNA alignments[86] (Figure 3(c)). SARSE was originally designed to detect and correct the structural misalignment of RNA alignments and was used in the context of a scaffolded DNA origami design framework as a scientific data editor to edit the bitmap shape and to modify the sequence of the scaffold and staples to match the design. The different perl scripts in this software package automate the five steps of Rothemund's scaffolded DNA origami design process as well as color coding the design and generating an atomic model of the design for further inspection.

In 2009, Williams et al. presented a three-dimensional editing tool for complex DNA structures called "Tiamat"[87] (Figure 3(d)) that addresses the challenge of efficiently modeling large DNA nanostructures using a data structure similar to that of GIDEON (i.e., base-centric) and a simplification algorithm for structure representation and rendering that depends on the distance of the camera lens from the structure; to tackle the efficiency problem, the authors (1) used simplified geometry of the structure while maintaining the same visual elements, and (2) represented helices by straight lines when the camera lens is away from the structure. They also provided the option for the user to switch to a more realistic view of the structure when needed. We note that while Tiamat can be used to design a wide range of structures, calculations of the physical distortion a structure will undergo in Tiamat has relatively little theoretical value. Williams et al. also provide a novel algorithm for sequence design. To avoid unwanted secondary structures in their designed sequences, the authors employed three constraints to the designed sequence: (1) unique sequence limit (i.e., shortest subsequence that must appear only once in the structure), (2) repetition limit (i.e., longest sequence of bases that can all be the same), and (3) GC percentage (i.e., minimum percent of G or C bases in the structure). Inspired by sampling algorithms, their designed algorithm then finds the answer to the optimization problem in which all of the above constraints are satisfied.

Douglas et al.[88] designed and developed a computer-aided design software, called "caDNAno", to automate the design process of scaffolded DNA origami to minimize cumbersome and error-prone tasks for the creation of complex 3D DNA nanostructures (Figure 3(e)). Using caDNAno, 3D nanostructures constrained to either a honeycomb or square framework can be designed. caDNAno is written in Python using a hierarchical data structure in which the lowest level of structural information is kept as a strand object. Each DNA strand is a data structure that stores end point information, index information, and connection information to other strands. All strands are kept in a container called "StrandSet", through which users can access different strands to create, destroy, resize, split, or merge them. A helix is formed in a scaffolded DNA origami when the scaffold and staple strands interact. Information about any two interacting strands (scaffold and one staple) in caDNAno is kept in a container called "VirtualHelix". This container is called virtual because a real helix is made up of multiple such VirtualHelix objects. Information about a group of strands connected via their end points (i.e., the physical DNA strand) is kept as an object of type "Oligo". The root of this hierarchical data structure is a "Document" class that tracks all lower level actions. Rules of base pairing in this model are base complementarity and basic geometry imposed by the design method of Rothemund.

Castro et al.[22] developed a computer-aided design tool for DNA origami, called "CanDo", that using a finite element method computes structural stability of caDNAno designs by employing continuum mechanics approximations (Figure 3(f)). CanDo models base pairs as two-node beam finite elements representing an elastic rod with experimentally determined geometric and material parameters. Following the caDNAno design steps, as an initial step, CanDo arranges all double helices linearly in space. Applying external forces to the helices, CanDo places rigid crossovers between the helices following the defined connectivity in caDNAno design. Using a relaxation step, it then finds incompatible connectivity of the design.

In 2014, Pan et al.[89] extended the CanDo framework to predict 3D structures consisting of multiway junctions constrained by DNA duplexes and to model topologically closed structures, including spherical ring-like origami objects (Figure 3(i)). The information about DNA sequence and its secondary structure is modeled by a directed graph in which nucleotides are represented as vertices and backbone connections, and complementary base pairings are represented as edges of the graph (i.e., base-centric data structure). Then, a graph traversal algorithm is used to identify junctions and duplex arm lengths, connectivity between junctions, and also initial position and orientation of junctions. Following this step, a finite element model is used to represent duplexes and multiway junctions with experimentally determined initial configurations. By performing an energy minimization procedure, the equilibrium 3D structure of the programmed DNA assembly is computed. Then using molecular dynamics (MD) simulations, they generate the 3D atomic structure and mechanical features associated with the predicted structure.

COMPUTATIONAL RNA ORIGAMI

As mentioned in the Introduction, Jaeger et al.[24] introduced Tecto-RNA units that self-assemble based on tertiary interactions (loop-receptor interactions) (Figure 3(k)). They checked each such RNA molecule with Mfold[94] to minimize the possibility of folding into alternative secondary structures. Mfold is a computational method for the prediction of minimum free energy pseudoknot-free RNA secondary structures using a dynamic programming algorithm with an underlying thermodynamics-based energy model. Each secondary structure is composed of different loop types, referred to as features. In Mfold, each feature is assigned a specific energy value, and the energy of a secondary structure is calculated as the sum of the energies of its features. Mfold takes as input an RNA sequence (i.e., a sequence of As, Gs, Cs, and Us) and predicts as output a secondary structure with minimum energy among all possible secondary structures. Atomic 3D models of the Tecto-RNAs were made manually using MANIP.[95] MANIP is a toolbox for designing three-dimensional models of RNA molecules assuming secondary structures of RNA molecules are known. Assuming no pseudoknotted base pairs (i.e., no crossing base pairs or kissing interactions) in theirTecto-RNAs, Jaeger et al. used the secondary structures produced by

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Mfold for their atomic 3D models. The work of Jaeger et al.[24] provides the first step for algorithmic self-assembly of RNA origami.

Chworos et al. presented algorithmic self-assembly of jigsaw puzzles with Tecto-RNAs[25] (Figure 3(l)). They used Tecto-RNA units with two hairpin loops joined by two stems with a total size of 11 nucleotides that poses a right angle between adjacent helices. With four such Tecto-RNA units, they assembled a Tecto-square with 2-fold pseudosymmetry. Then, through the addition of sticky tail connectors to Tecto-RNAs, they generated five different Tecto-squares with sticky ends capable of self-assembly similar to a jigsaw puzzle. Similar to the work of Jaeger et al.,[24] Tecto-RNA sequences were optimized using Mfold. The tail connectors (6 base pairs long) were designed using ssRNAdesigner program, an in-house software, such that they did not form a base pair with themselves or the Tecto-RNA of which they were a part.

Nasalean et al.[26] used H-shaped Tecto-RNA units to introduce control over directionality of self-assembly of these structural units (Figure 3(m)). Although we consider the study of directionality of RNA self-assembly as a computational achievement, the authors did not design or implement new methodology or software tools to evaluate/complement their experimental study; instead, similar to the work of Jaeger et al.[24] and Chworos et al.,[25] they used a combination of Mfold[94] and MANIP[95] software tools.

Bindewald et al.[93] created the first computational tool, called "NanoTiler", for RNA nanostructure design that uses known RNA structural motifs as building blocks to construct nanostructures (Figure 3(n)). NanoTiler is an editor tool that receives a 3D structure and a database (RNA junctions[96]) as input and provides a list of possible RNA sequences as output. In more detail, after determination of the desired structure in terms of a 3D graph, the user inputs the coordinate file corresponding to this graph to NanoTiler, which searches the RNA junctions database to identify possible building blocks (known RNA motifs) for this graph with the smallest fitting error. With a set of possible junctions, NanoTiler randomly chooses each junction and fills the gap in the structure with helices to produce the best fitting sequence. After this point, users can optimize the sequence further as an optional step. Molecular mechanics and molecular dynamics are then used to further refine the structure. This process can be performed in iteration to ensure the necessary optimization.

We note that, although there are numerous publications on sequence optimization and design for single RNA molecules,[97−112] in this paper, we focus on methods that were designed and used for generating nucleic acid origami structures. We refer interested readers to some comprehensive reviews on these topics.[113−115]

In 2014, Geary et al.[31] introduced a general design strategy for the generation of RNA tiles to be used in self-assembly in both heated annealing and cotranscriptional folding (Figure 3(o)). Their computational framework[116] uses a parametrized model for RNA helices to determine geometry and base pair spacing as well as the curvature resulting from crossovers between multihelix structures. Their proposed simplified model, "P-stick", is based on the following five parameters with experimentally assigned values:[117,118] (1) base inclination (relative to the helix axis), (2) rise between bases, (3) helix radius, (4) helix angle across the minor groove, and (5) helicity in base pairs per turn. They also demonstrated general strategies for the design of folding pathways using kissing interactions and their newly introduced dove-tail seams that could be extended to other tertiary motifs.

MOLECULAR DYNAMICS AND COARSE-GRAINED SIMULATIONS

Although several software interfaces[85,88] have been developed to design and predict the 3D conformation of DNA origami structures over the past decade, the atomistic simulations considering the molecular interactions underlying this process are still sparse. Stability and self-assembly of DNA origami objects have a more complex nature and involve hydrogen bonding, electrostatic, hydrophobic, solvent-mediated, and van der Waals forces. Knowledge of these interactions is essential to understand and control the formation of self-assembled nanostructures to pave the way for tailored materials according to the desired application. All-atom molecular dynamics (MD) methods can be used as a complement to less accurate but more computationally efficient methods based on continuum mechanics[119] to determine the overall geometry and structural stability with ultimate temporal and spatial resolution. By following the trajectories of all atoms, MD methods can provide complete structural information about each and every nucleotide of a DNA origami object under experimental conditions (i.e., in the presence of water and ions), local mechanical stresses, and their microscopic origins. Moreover, for a proven DNA origami design, an all-atom MD simulation can be used as a cost-effective replacement for cryo-EM reconstruction.

In 2013, Yoo and Aksimentiev[90] used MD simulations to report the first full atomic precise portrait of a DNA origami in solution. They modeled two rectangular structures with different lattice structures along with an origami with a bended structure (Figure 3(g)). They reproduced experimentally known structural properties of DNA origami objects and also provided predictive information that is currently not accessible experimentally. They showed that DNA origami structures undergo considerable temporal fluctuations on both local and global scales, which affects global mechanical properties, such as bending rigidity. In another study, it was shown that these structural fluctuations could be significantly enhanced in the case of the DNA origami nanopores due to the presence of a strong electric field.[120]

All-atomic modeling approaches are computationally too expensive to study the hybridization and self-assembly of DNA origami at high concentrations due to the long time scales, larger system sizes, and rarity of events. By employing less detailed "coarse-grained" (CG) models, which are based on the integration of a large number of degrees of freedom into a few coarse grained beads, the time scale accessible to simulations of DNA origami objects can be significantly extended. In contrast to the MD method, CG models often neglect the solvent molecules, implying a compromise between accuracy and computational efficiency. In recent years, many CG models of DNA have been developed to reproduce various properties of double stranded DNA (dsDNA),[121−130] but only a few of them are well-suited to studying the self-assembly processes associated with DNA nanotechnology. In 2013, Doye et al. used an oxDNA coarse-grained model to reproduce the structure of DNA nanotetrahedrons, three-armed star motifs, and origami structures, such as "smiley face", made up of 6196 bases.[91] oxDNA was first developed in 2010[131] with promising abilities to exhibit the physical properties that are most relevant to applications associated with DNA nanotechnology, such as a

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realistic description of the structure, thermodynamics, and mechanics of both dsDNA and single-stranded DNA (ssDNA). In the oxDNA model, strands of DNA are represented by strings of nucleotides. Each nucleotide is modeled as a rigid body with three interaction sites: the hydrogen bonding, stacking, and backbone sites, interacting through effective potentials (Figure 3(h)). The oxDNA model was also applied to study DNA nanodevices like nanotweezers[131] and walkers.[132] One drawback of the model is that it does not have sufficient structural detail to reproduce experimentally observed right-handed chirality in 4-way junctions. At first, oxDNA was only parametrized for high NaCl concentration [0.5 M][91] but recently was improved to include a range of salt-concentrations, including those corresponding to physiological conditions.[133]

In 2014, Reinhardt et al.[92] performed Monte Carlo (MC) calculations to study the self-assembly behavior of experimentally observed DNA bricks (Figure 3(j))[17] comprising approximately 1000 types of DNA strands. They modeled the DNA strands as lattice tetrahedra with attractive patches, the interaction strengths of which were computed using a standard thermodynamic model. They reported an optimum temperature range where self-assembly of the bricks is successful, above which the monomer phase is entropically favored, whereas below it large aggregate structures forms. By this model, they portrayed the basic physics of self-assembly, which supported the suggestion of Ke et al.[17] that initial structure growth is a slow process.

Fully atomistic MD of several folding pathways of a short RNA hairpin[134] and tetraloops[135] provide examples of the time scale limit of what is currently possible (time scales on the order of nanoseconds). Methods that combine fully atomistic representations with hierarchical MC sampling were used to study the effect of mutations on conformational freedom of a nanosquare composed of four tRNAs as structural building blocks of tRNA-based nanotechnology.[136] To capture rare events, such as the breaking of the base pairs or the self-assembly and formation of large RNA structures, we needed simulations with longer time scales than the reach of current simulations. For longer time scales to be accessed, a more coarse-grained method could be helpful. Several coarse-grained models have been developed in recent years.[137−147] These models, parametrized either through structure or thermodynamics-related information, are mostly used to predict the folded structure of RNA. Although efforts have been put forth to favor the thermodynamics parameter choices, they have not presented enough verification of thermodynamics and mechanical properties for several different systems.

Given that oxDNA was successfully used to model DNA nanotechnology systems, Sulc et al. recently presented a new, off-lattice model for RNA, oxRNA,[148] based on the oxDNA model of DNA. In this model, each RNA nucleotide is replaced with a single rigid body including multiple interaction sites. The interactions between rigid bodies are parametrized to reproduce structural, mechanical, and thermodynamic properties of large RNA structures. Multiple strands of RNA (thousands of base pairs and for the assembly of systems of up to hundreds of base pairs) can be involved in simulations based on this model and the coarse-graining level aimed to retain the relevant physics for RNA hybridization and the structure of single- and double-stranded RNA.

oxRNA was tested for a range of nanotechnological and biological settings to explore applications including the folding thermodynamics of a pseudoknot, formation of a kissing loop complex, structure of a hexagonal RNA nano-ring, unzipping of a hairpin motif,[148] and a toehold-mediated RNA strand displacement reaction.[149] Although oxRNA can semi-quantitatively reproduce a wide range of thermodynamic data, there are properties like the melting temperature of certain motifs that have larger deviation from experimental data in oxRNA than in oxDNA. The authors ascribed the difference between the coarse-graining of oxDNA and oxRNA to two facts:[149] first, RNA has more complex behavior than DNA, making it harder to coarse-grain; and second, a general phenomenon called the "representability problem".[150] In this case, the stronger the fitting to one set of input data (i.e., structure of DNA), the larger the errors in other quantities (i.e., thermodynamics properties). For further progress to be made, an extension of the single nucleotide model of RNA to a higher-level model with a better representation of both thermodynamics and structure could be useful.

CURRENT CHALLENGES AND FUTURE DIRECTIONS

Although both DNA and RNA origami have come a long way, they are both relatively new research topics, particularly in terms of computational modeling and simulations. By comparing Figures 1 and 3, we can see a large gap between the development of research in DNA and RNA origami as well as the lag of computational research in these two fields. In this paper, we aimed to provide a review of computational achievements in nucleic acid origami, their importance in experimental design, and to promote research in these fields. In this section, we provide the current challenges in these fields and possible directions for future research.

There are important experimental and computational challenges that need to be addressed before applications of nucleic acid origami become a reality. First and foremost are the current limitations related to cost and accuracy of synthesized DNA strands.[34,151] Utilizing new, fast, accurate, and cost-effective methods for synthesizing DNA strands, we can fancy new supramolecular origami structures in the future. Current scaffolded origami based methods use an existing long DNA strand (M13) that may not be the optimal sequence for different origami structures. Advances in DNA synthesis would provide an opportunity to use specifically optimized sequences for every different structural design. Additionally, currently used staple DNA sequences are in the range of 18 to ∼50 bases due to stability and cost. A systematic study of the relationship between staple length, structural stability, and folding quality is currently lacking.

Current methods for scaffolded DNA origami require an excess amount of staple strands, and although structural yield in single-layer origami can be as high as 100%, in multilayer origami, the objects yield may drop to 5−20%.[22] Thus, another opportunity for improvement is in designing new methods that improve structural yield for complex origami structures that do not need excess staple strands.

New methods for origami structure analysis are also needed. For the evaluation of structural yield and quality, one method that is currently used is AFM imaging. In this method, origami structures are attached to a mica film for imaging. It is not yet clear whether some of the distortion seen in an AFM image is the result of faulty production or distortion caused by mica attachment. This challenge is even more pronounced for RNA origami structures due to their RNA specific characteristics

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(being a soft, less chemically stable medium compared to DNA).

Furthermore, attaching origami structures to films causes 2D chirality of the objects. Development of new methods to control attachment of origami structures to films in only one orientation can improve the creation of well-defined origami assemblies.

Another possible improvement to scaffolded origami design is exploring the sequential addition of staple strands to folding pots to guide the folding pathway in the desired folding direction and to avoid kinetic traps.

For origami structures to be used in therapeutic applications, more application-specific challenges need to be answered. DNA origami structures need to be inserted into cells or new technology needs to be developed to synthesize DNA strands within cells. In the case of RNA origami where RNA can in fact be synthesized inside living cells, researchers need to address how to synthesize the exact optimal RNA strand in a cell and examine the cells' response to large origami structures. Furthermore, degradation time for RNA origami structures needs to be carefully studied so that origami structures can be created, activated, and removed/degraded in a timely manner.

Research on computational nucleic acid origami has even larger steps to take. As mentioned before, to design a computationally tractable method, we cannot currently use all existing information about molecules (as MD simulations, for example, are not great solutions due to their high computational cost). Therefore, pivotal parameters for specific applications need to be identified and efficient algorithms need to be designed based on these parameters to correctly guide experimental design. Some improvement in parameters might be obtained by focusing on sequence details and modeling interhelical electrostatic repulsion and major and minor groove details.

Current design methods mainly focus on using known RNA motifs and junctions to create desired structures. Another strategy can be to focus on the secondary structure instead of the 3D structure for the purpose of design. By secondary structure, we refer to the structure defined by RNA base pairs and unpaired bases. With this definition, we consider pseudoknotted base pairs (i.e., base pairs that form kissing interactions) as part of the secondary structure. While abstraction to secondary structure reduces the complexity of design, there is evidence that secondary structure sheds light on the 3D structure,[69] and there are existing programs to convert secondary structure to three dimensions (see, for example, RNA2D3D[152]). One structure design strategy can be to combine sequence design and secondary structure prediction in an iterative way to optimize the structure. For this purpose, focus needs to be on both fronts, i.e., the sequence design problem as well as the secondary structure prediction problem, where both cases should be extended to include common pseudoknotted structures.

While throughout the literature researchers have tried to use secondary structure prediction methods, they mainly used Mfold[94] to check for desired structures.[26,27] Mfold predicts the secondary structure of pseudoknot-free structures, but it is not capable of predicting possible pseudoknotted structures that may form from inter- and intramolecular interactions. Geary et al.[31] designed their RNA sequences using NUPACK software,[153] which includes pseudoknotted structures. However, kissing interactions, which are crucial motifs in RNA nanotechnology, are not in the class of structures predicted by NUPACK.

The best-suited algorithms for the purpose of origami design are, in our minds, methods with underlying energy models that resemble origami folding pots and consider thermodynamics and possibly kinetics of the assembly. For this reason, we suggest focusing on methods based on free energy minimization (examples include 154−156). Methods based on free energy minimization use a dynamic programming algorithm to find the structure with the lowest energy among the pool of possible structures. These methods can be used to design synthetic strands for different applications in biotechnology. We acknowledge that there are limited parameters available for pseudoknot formation and believe improvements in energy models is a step that benefits both the nanotechnology community as well as the general bioinformatic community. While methods based on minimum free energy calculations can output one or a few output structures (i.e., suboptimal structures), they can be converted in a straightforward way to partition function calculation methods to provide more insight into the structure, including base pair probabilities and melting temperature. To use such methods, one should address how to represent multiple nucleic acid strands. When predicting the structure of two interacting strands, one possible option is to simply attach the two strands together and treat them as a single strand while treating the gapped region between the two strands as a special type of loop. However, when the number of interacting strands increases to more than two, other problems, such as possible symmetry effect of the predicted structure,[153] need to be answered as well.

Another concept that might be important in the design of RNA origami structures is consideration of the hierarchical folding of RNA molecules. Recently, algorithms have been developed[157,158] for the prediction of pseudoknotted secondary structures of RNA molecules that implement hierarchical folding of RNA molecules and that are less computationally expensive than other methods based on minimum free energy determination.

There has been significant effort in addressing the challenges of designing the structure of a single complex of one or more interacting nucleic acid strands.[97−112] However, by ignoring the concentration of the complex and desired and off-target structures, sequences optimized to fold into a desired structure may fail to do so in a test tube setting. Wolfe and Pierce have recently described an algorithm that addresses these problems.[159]

MD calculations equipped with a physically correct description of interatomic interactions (force fields) and adequate computational power should be able to predict and control the physical behavior of nucleic acid origami in nanotechnology applications. Although force fields have improved substantially for nucleic acids[160−167] in recent years, the accuracies of the force field parameters and the models of solvation free energy must be improved to reach a level of accuracy that is sufficient for predictive RNA and DNA self-assembly simulations. Improving coarse-grained simulations is yet another direction for future improvement. The description of nucleic acid coarse-grained models on the level of a single base could be extended to more complex models to achieve the next level of accuracy. The electrostatic behavior of DNA and RNA is potentially very complex, particularly for systems with divalent cations. In most CG models, the electrostatics are treated in a basic fashion. In fact, an understanding of the

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consequences of electrostatics for the stability of systems like nucleic acid origami would be interesting in its own right.

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Notes

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