

# In Silico Design and Enzymatic Synthesis of Functional RNA Nanoparticles

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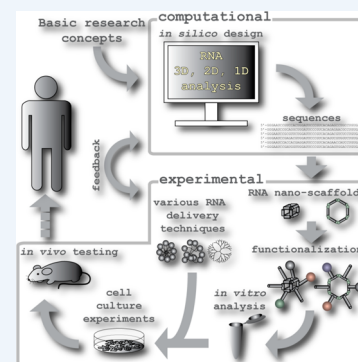
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## S Supporting Information

**CONSPECTUS:** The use of RNAs as scaffolds for biomedical applications has several advantages compared with other existing nanomaterials. These include (i) programmability, (ii) precise control over folding and self-assembly, (iii) natural functionalities as exemplified by ribozymes, riboswitches, RNAi, editing, splicing, and inherent translation and transcription control mechanisms, (iv) biocompatibility, (v) relatively low immune response, and (vi) relatively low cost and ease of production. We have tapped into several of these properties and functionalities to construct RNA-based functional nanoparticles (RNA NPs). In several cases, the structural core and the functional components of the NPs are inherent in the same construct. This permits control over the spatial disposition of the components, intracellular availability, and precise stoichiometry.

To enable the generation of RNA NPs, a pipeline is being developed. On one end, it encompasses the rational design and various computational schemes that promote design of the RNA-based nanoconstructs, ultimately producing a set of sequences consisting of RNA or RNA–DNA hybrids, which can assemble into the designed construct. On the other end of the pipeline is an experimental component, which takes the produced sequences and uses them to initialize and characterize their proper assembly and then test the resulting RNA NPs for their function and delivery in cell culture and animal models. An important aspect of this pipeline is the feedback that constantly occurs between the computational and the experimental parts, which synergizes the refinement of both the algorithmic methodologies and the experimental protocols. The utility of this approach is depicted by the several examples described in this Account (nanocubes, nanorings, and RNA–DNA hybrids). Of particular interest, from the computational viewpoint, is that in most cases, first a three-dimensional representation of the assembly is produced, and only then are algorithms applied to generate the sequences that will assemble into the designated three-dimensional construct. This is opposite to the usual practice of predicting RNA structures from a given sequence, that is, the RNA folding problem. To be considered is the generation of sequences that upon assembly have the proper intra- or interstrand interactions (or both). Of particular interest from the experimental point of view is the determination and characterization of the proper thermodynamic, kinetic, functionality, and delivery protocols. Assembly of RNA NPs from individual single-stranded RNAs can be accomplished by one-pot techniques under the proper thermal and buffer conditions or, potentially more interestingly, by the use of various RNA polymerases that can promote the formation of RNA NPs cotranscriptionally from specifically designed DNA templates. Also of importance is the delivery of the RNA NPs to the cells of interest *in vitro* or *in vivo*. Nonmodified RNAs rapidly degrade in blood serum and have difficulties crossing biological membranes due to their negative charge. These problems can be overcome by using, for example, polycationic lipid-based carriers. Our work involves the use of bolaamphiphiles, which are amphipathic compounds with positively charged hydrophilic head groups at each end connected by a hydrophobic chain. We have correlated results from molecular dynamics computations with various experiments to understand the characteristics of such delivery agents.



## INTRODUCTION

RNA nanobiology has its roots in a talk given by Nobel laureate Richard Feynman presented in 1959 entitled “There is plenty of room at the bottom” (<http://calteches.library.caltech.edu>) where he defined the field of nanotechnology as the making of new materials by the direct manipulation of atoms and

molecules. At the molecular level, van der Waals forces, solvation, and hydrogen bonding are more important compared

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with forces of everyday life such as gravity and inertia. Nanotechnology may thus lead to the creation of materials with novel properties and functions.

Seeman and co-workers applied this concept to the fabrication of novel DNA-based nanomaterials through DNA self-assembly, which resulted in numerous DNA based nanoobjects for various applications.<sup>1–7</sup> The recently developed by Rothemund “DNA origami” technique<sup>8</sup> facilitates the straightforward design of larger 2D and 3D shapes.<sup>9–11</sup>

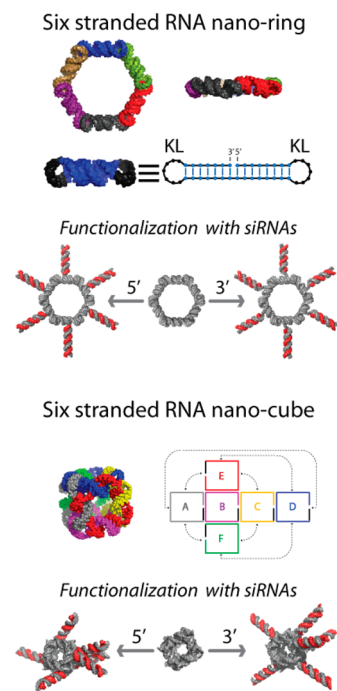
The same concept can be also applied to the domain of RNA nanobiology, which might be defined as the rational design and experimental assembly of multistranded RNA constructs that simultaneously incorporate various functionalities, some of which have the potential for therapeutic purposes.<sup>12–14</sup> Using natural or artificially selected RNA motifs and modules,<sup>15,16</sup> RNAs can be programmed to form a wide variety of compact and stable artificial 3D nanostructures (RNA NPs)<sup>17,18</sup> suitable for a broad range of clinical and nanotechnological applications.<sup>19–22</sup> Therapeutic nucleic acids, proteins, or small molecules can be individually attached (using different techniques<sup>23</sup>) to the programmed RNA monomers, which form RNA NPs. The assembly of the monomers brings the desired functionalities together, thus providing precise control over their topology, composition, and modularity. The use of functional RNA NPs *in vivo* provides a higher concentration and desired stoichiometry of therapeutic moieties locally. For therapeutic nucleic acids, RNA interference (RNAi<sup>24</sup>) is progressively investigated for possible treatment of various diseases through the exogenous introduction of short synthetic RNA duplexes called small-interfering RNAs (siRNAs).<sup>25</sup> Besides siRNAs (or micro-RNAs), several other promising therapeutically potent RNA classes such as antisense RNAs, aptamers, and ribozymes are available.<sup>13</sup> Simultaneous use of multiple different RNA therapeutics is expected to have significant synergistic effects.<sup>13</sup> One well-known example is a combinatorial RNAi (co-RNAi) used for highly effective simultaneous multiple gene suppression preventing the possibility of mutation-assisted escape from RNAi (e.g., in the case of HIV).<sup>26</sup>

Our recent work in RNA nanobiology established two orthogonal strategies for RNA NP (for example, nanocubes and nanorings) design and production.<sup>17,19,27,28</sup> As illustrated in Figure 1, extension of either the 3'- or 5'-end of each of the nanoscaffold strands allows embedding of different functionalities. For example, nanocubes and nanorings were modified to package multiple siRNAs that simultaneously target different regions of the HIV-1 genome,<sup>19</sup> thus limiting viral escape due to mutations. Most importantly, the precisely controlled therapeutic composition of RNA NPs can be easily altered by swapping the functionalized monomers.

In this Account, the main strategies for computational design and experimental production of these two types of RNA NPs are discussed.

## ■ NANOSTRUCTURE MODELING STRATEGIES

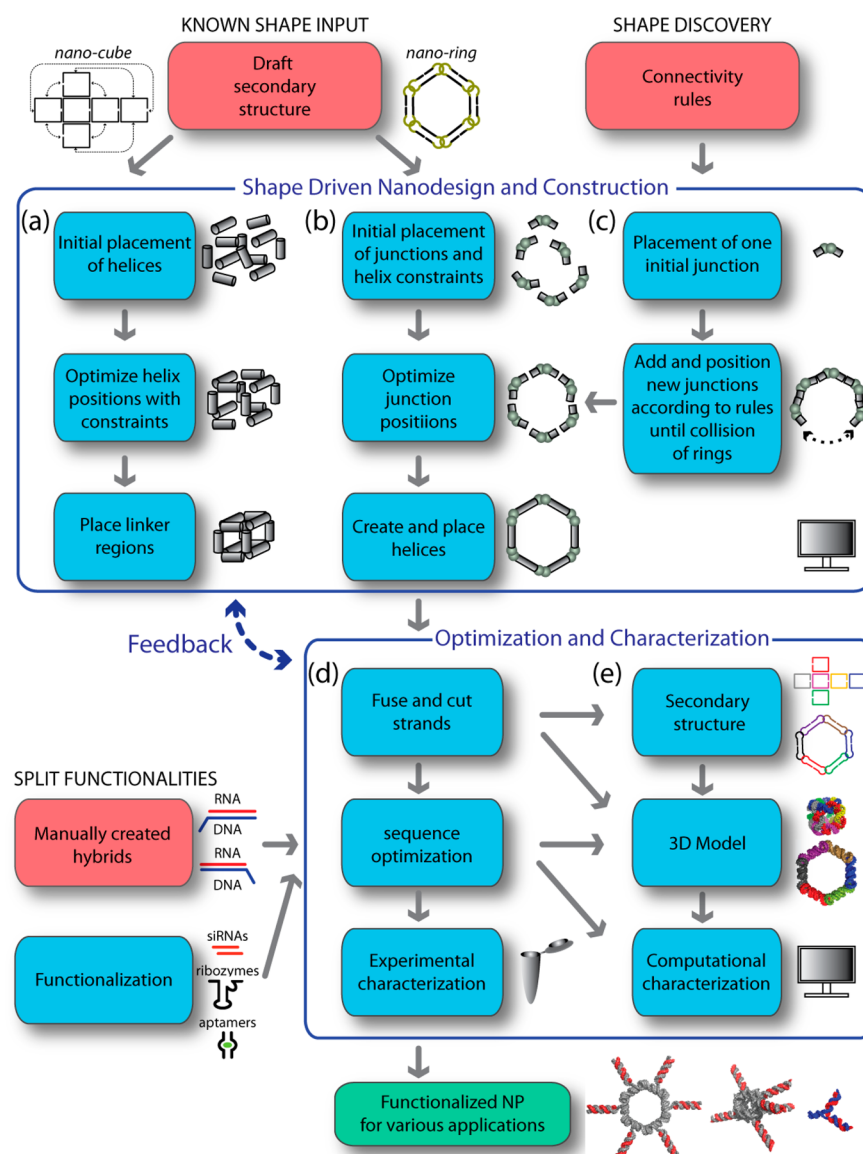
While no single strategy can serve every RNA nanoscale design process, we have identified two general approaches, one based on known shape input and the other on shape discovery (Figure 2). Both combine structural building blocks from a database or ones generated *de novo* with linker structures, such as helices or single strands, to produce a 3D nanostructure. These approaches can be divided into three more specific strategies, which we implemented as computer-aided pipelines.



**Figure 1.** Functionalization of RNA-based nanoscaffolds (nanorings and nanocubes) with therapeutic siRNAs through the extension of nanoscaffold strands (either 5'- or 3'-side). Adapted in part with permission from refs 17 and ref 19. Copyright 2010 and 2011 Nature Publishing Group.

The first two strategies produce structures satisfying a specified input shape. In the first strategy (Figure 2a), helices are placed in a 3D workspace, and their positions are optimized and then connected with single-stranded (ss) linkers. Effectively, junctions between multiple helices are created *de novo* in this case.<sup>17,29</sup> The second strategy (Figure 2b) is driven by an input set of junctions, that is, RNA structure fragments, including internal, multibranch, and kissing loops, with short helical stubs emanating from them, such as those stored in our RNAJunction database (Figure 3).<sup>30</sup> First, junctions are placed in a 3D workspace, followed by creation of linker helices. The third strategy (Figure 2c) combinatorially produces a set of closed-shape structures (shape discovery) given a set of junctions and rules for their use (number of junctions to be used in one structure, linker helix length limits, etc.).<sup>31</sup> A full 3D nanostructure model may be further functionalized with, for example, siRNAs, ribozymes, aptamers, or split functionalities (RNA–DNA hybrids described later). The resulting 3D structure is used to derive the final secondary structures for use by programs that optimize the sequence or sequences guaranteeing their correct self-assembly (Figure 2d,e).

While there are a variety of RNA 3D modeling programs,<sup>32,33</sup> we mostly use our NanoTiler and RNA2D3D programs for nanostructure modeling (Figures 4).<sup>31,34</sup> The user can interact with NanoTiler via a graphical interface (Figure 4a) or scripting language.<sup>29,35</sup> One of the capabilities of the program is to optimize motif placements and helix distortions in order to achieve structure closure. Once the structural design issues are resolved, a Web server called NanoFolder can perform sequence design to generate RNA sequences that are predicted to self-assemble into RNA NPs.<sup>31,36</sup> After sequence optimization, NanoTiler can be used again to perform mutations on the



**Figure 2.** Flow-chart depicting the main modeling strategies for design of functional nanostructures.

initial “dummy”-sequence-based nanostructure to create a 3D model for further characterization.

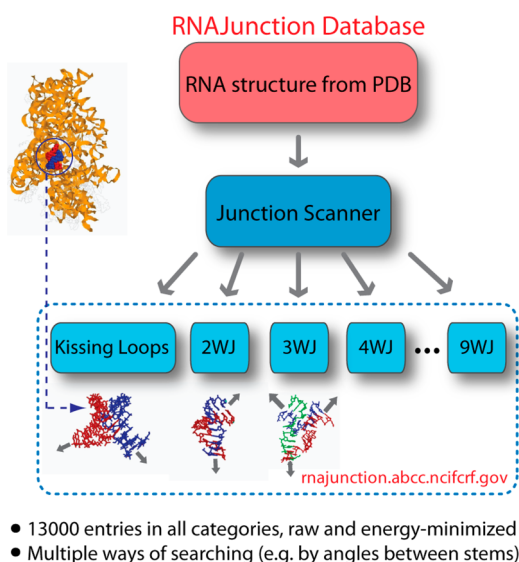
RNA2D3D is an interactive program that takes as input an RNA sequence with a corresponding secondary structure descriptor, including pseudoknots (Figure 4b).<sup>34</sup> The program also allows one to define interactions between multiple building blocks and rapidly generates an approximate 3D model (including mesh-like structures), leaving further refinements to the user.<sup>34,35</sup> The full 3D model or its user-defined parts can be subjected to energy minimization and short molecular dynamics runs in order to “clean-up” imperfections. Structural motifs from databases, such as RNAJunction or the PDB, for example, can be substituted in place of equivalent model subdomains. Please, refer to the Supporting Information for more details on NanoTiler and RNA2D3D.

## ■ NANOSTRUCTURE CHARACTERIZATION

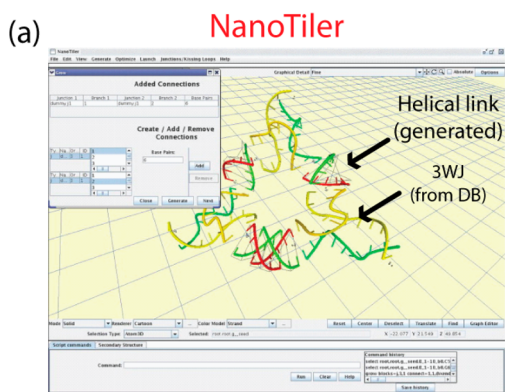
Characterization of nanostructure flexibility and dynamics plays an integral role in the modeling process and may even be a way to achieve structure closure in modeling of rings.<sup>35</sup>

RNA tectosquares are modular designs in which four monomers interact with each other via kissing loops to form individual squares and via ssRNA tails to link multiple squares into programmable meshes.<sup>16</sup> We applied RNA2D3D and NanoTiler to build the models and explore their closure, based on molecular dynamics (MD) simulations of overlapping tectosquare fragments.<sup>35</sup> The MD results underscored the influence of magnesium ions (required for assembly) on the structural geometry changes, aided in modeling of a closed ring structure, and demonstrated that the molecular model generated with NanoTiler fell within the range of structures obtained by MD.

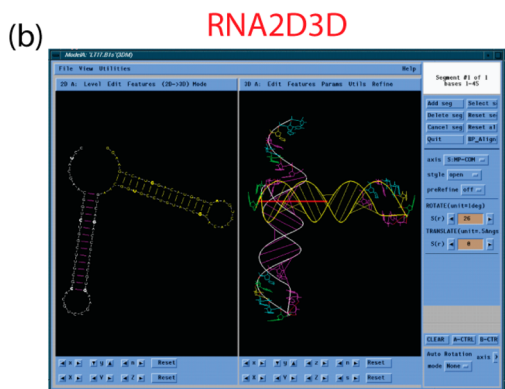
Because MD simulations are time-consuming, we evaluated a faster approach to generating potential dynamic states of nanostructures by employing an anisotropic network model (ANM).<sup>37</sup> An ANM represents a molecule as a network of nodes connected by springs providing the potential energy. It can predict directions and the relative magnitudes of the major collective motions of a structure, indicating, for example, the closure potential in the ring structures or distortion limits of



**Figure 3.** Flow-chart depicting RNAJunction database working principles.



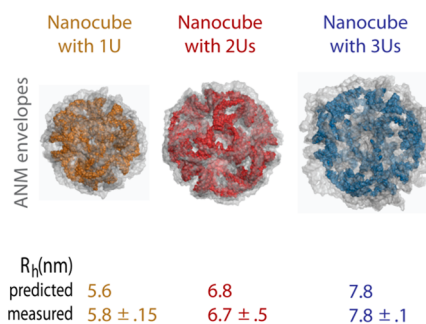
- Builds nanostructures via combinatorial search among junctions and connectivities
- GUI and/or scripting language
- Junctions and KLs from DB or created *de novo*
- 3D nucleotide mutations



- 3D models from sequence and 2D information
- GUI-based interactive modeling
- Incorporates structure fragments from DBs
- Limited nanostructure modeling

**Figure 4.** Screenshots of an interactive window of (a) NanoTiler and (b) RNA2D3D.

nanocages, such as our nanocubes. We recently presented a full modeling process for three variants of a nanocube, starting with the NanoTiler-built models, through the characterization of the nanostructures' flexibility with the aid of ANM simulations.<sup>29</sup> The apparent size changes due to the distortions of the cubes predicted by ANM brought the computational and the experimental (DLS) nanoparticle size measurements into agreement (Figure 5), suggested reasons for the measured melting temperature differences for the cube variants, and offered more insight into the observed assembly yield differences.



**Figure 5.** Predicted and measured dimensions of the nanocubes with 1U, 2U, and 3U single-stranded corner linkers. Adapted in part with permission from ref 29. Copyright 2013 Elsevier.

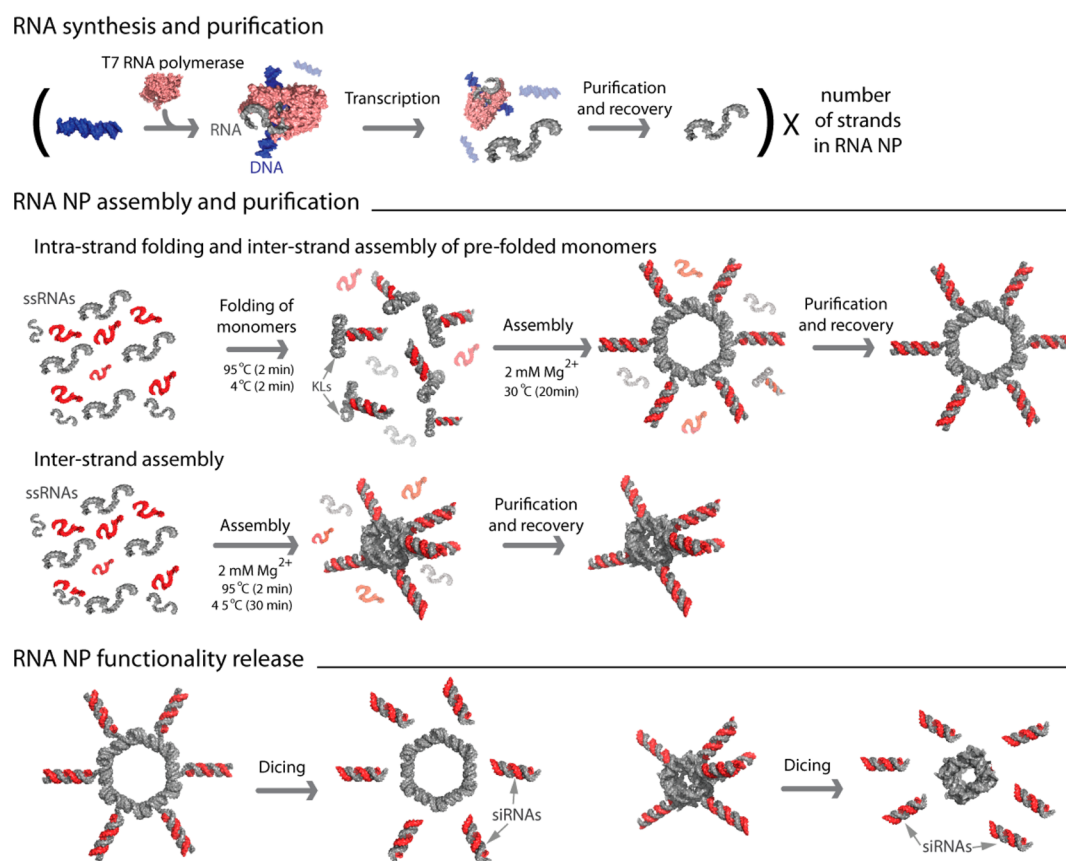
## ■ MULTISTRAND SECONDARY STRUCTURE PREDICTION AND SEQUENCE DESIGN

Design of RNA NPs is based on the ability to predict the pairing interactions of a given RNA nucleotide sequence. However, since RNA NPs are frequently multistranded, this problem typically goes beyond the classic ssRNA secondary structure prediction. From a computational point of view, these RNA NPs are frequently highly pseudoknotted entities, because their base pair interactions are, if displayed in a circle diagram, non-nested.<sup>36</sup>

The minimum free energy structure for a given set of RNAs can be predicted by several programs.<sup>38–40</sup> Our NanoFolder program provides secondary structure prediction for multiple RNAs with arbitrary pseudoknots and a general framework for the prediction of multistranded complexes, without limitations in terms of pseudoknot complexity.<sup>36</sup>

An alternative approach of ours using RNA–DNA hybrid duplexes, which have thermodynamic properties that differ from those of pure RNA–RNA or DNA–DNA interactions, creates a challenge for computational algorithms, which must account for the three possible cases of competing RNA–RNA, DNA–DNA, and RNA–DNA interactions.<sup>41–43</sup> We recently demonstrated that RNA–DNA hybrids can be designed computationally to allow for a controlled release of multiple siRNAs.<sup>41</sup>

Determining the sequence of nucleotides that would fold into a given structure is not a trivial problem, due to imperfect thermodynamic rules and additional constraints related to sequence synthesis or three-dimensional folding requirements. The program RNA-SSD uses a stochastic local search algorithm for identifying sequences with minimal differences between the predicted and the desired secondary structure.<sup>44,45</sup> INFO-RNA utilizes a dynamic programming algorithm as a first computational stage, followed by a local stochastic search to further



**Figure 6.** Steps of RNA NP production and release of siRNAs through dicing. Adapted in part with permission from ref 19. Copyright 2011 Nature Publishing Group.

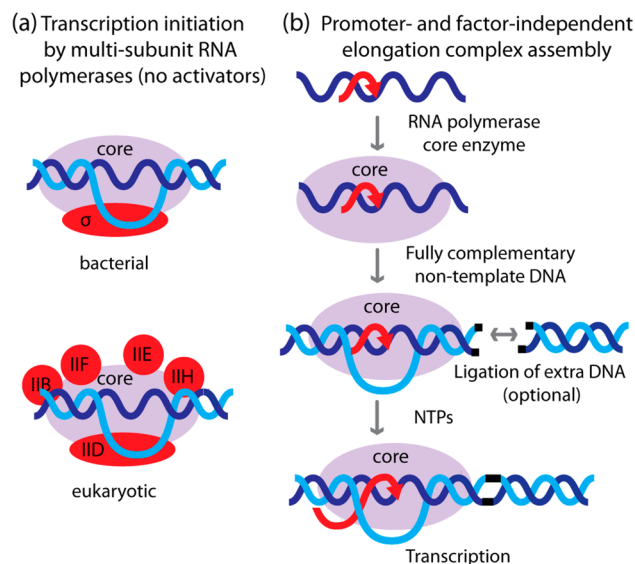
optimize the set of sequences.<sup>46</sup> NUPACK performs sequence design using a score that reflects the difference with respect to the target secondary structure for a predicted ensemble of RNA structures.<sup>39,40</sup>

The NanoFolder sequence design approach is based on a Monte Carlo search in sequence space, utilizing a scoring function that includes a variety of terms.<sup>36,47</sup>

## ■ ENZYMATIC PRODUCTION OF RNA NANOPARTICLES

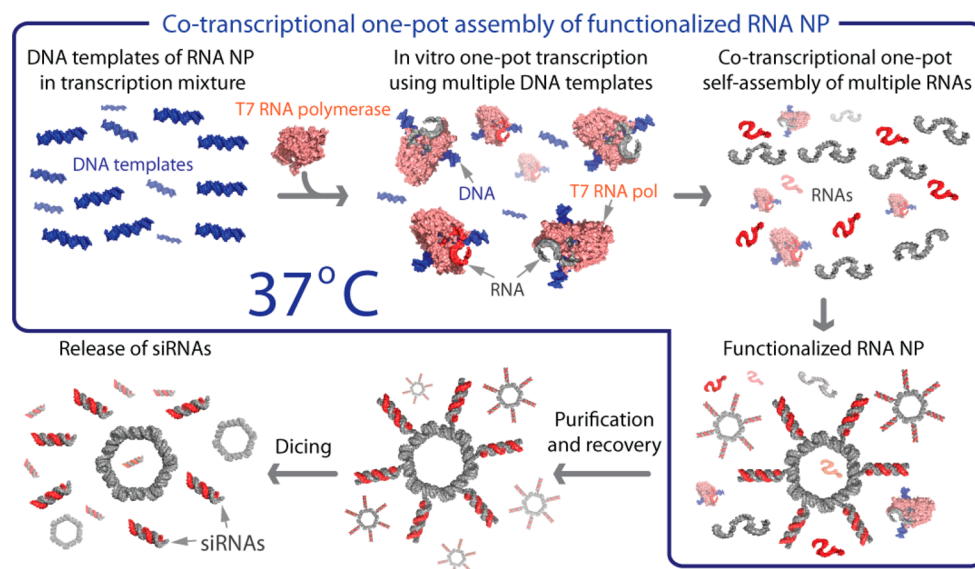
Currently, RNA NP production includes several steps (Figure 6): synthesis of individual strands, their purification and recovery, stoichiometric mixing, thermal denaturation and renaturation, assembly of RNA NPs, and further purification. Thermal renaturation and assembly conditions depend on the NP design approach and often have to be optimized for each type of RNA NP (e.g., nanocubes and nanorings). This together with the present length limitations (>70 nts) on chemical synthesis of RNA chains, emphasizes the importance of enzymatic RNA NP synthesis by *in vitro* transcription (IVT) in biotechnology and nanomedicine. Below we summarize the current state and perspectives of IVT methodology development for the experimental pipeline.

RNA polymerases (RNAPs) from bacteriophages T7 and SP6 are commonly used for RNA production by IVT.<sup>48</sup> Bacteriophage RNAPs (Figure 7) are single-subunit enzymes that do not require any additional factors for accurate transcription initiation on their short (<30 bp) promoters. Transcription is fast (100–200 nt/s)<sup>49</sup> and multiple transcripts as short as 30 nt<sup>50</sup> or as long as 30 kb<sup>48</sup> are obtained from a

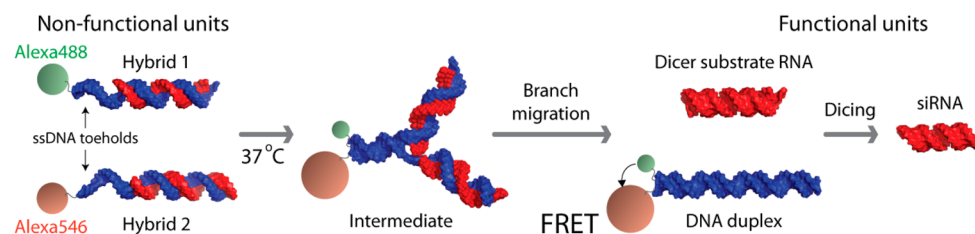


**Figure 7.** Transcription with multisubunit RNAP *in vitro*: (a) promoter-dependent initiation and (b) promoter-independent assembly of the elongation complex (RNA is red; DNA is blue).

single DNA template. High transcription efficiency allows production of chemically modified RNAs that are essential for a variety of applications.<sup>51,52</sup> The availability of mutants that decrease substrate specificity of T7 RNAP further broadens the range of chemically modified NTPs used as substrates for RNA synthesis.<sup>53</sup> Recently, we developed a generalized *in vitro* methodology for one-pot cotranscriptional assembly of differ-



**Figure 8.** Enzyme assisted one-pot cotranscriptional production of functional RNA NPs. Adapted in part with permission from ref 52. Copyright 2012 American Chemical Society.



**Figure 9.** Simultaneous activation of FRET and RNAi upon reassociation of RNA–DNA hybrids.

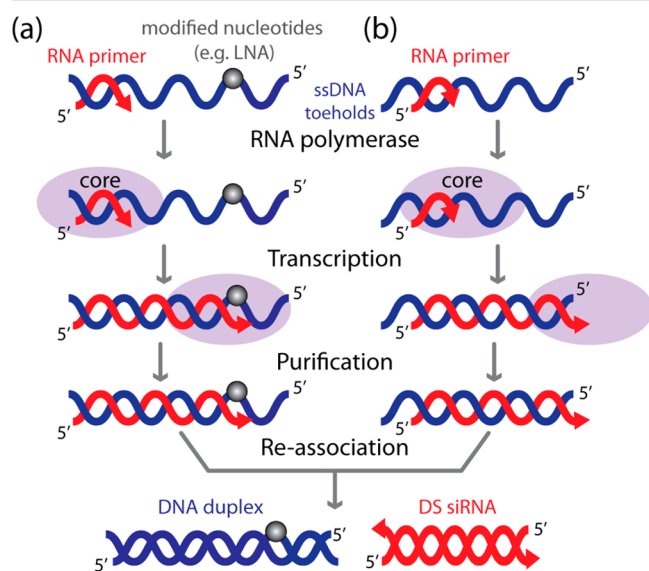
ent RNA NPs<sup>17,52,54</sup> with some NPs carrying up to ten siRNAs for co-RNAi.<sup>52</sup> IVT was performed with a mixture of DNA templates carrying specific T7 RNAP promoters and encoding RNAs programmed to form NPs (Figure 8). Relatively high assembly yields and experimental simplicity were successfully achieved.<sup>17,52</sup> Incorporation of chemically modified nucleotides (e.g., 2'-F-dUMPs) into the functional RNA NPs increases their resistance to nuclease degradation in blood serum and is achieved by IVT in the presence of Mn<sup>2+</sup>.<sup>52</sup>

The use of multisubunit RNAPs for preparative production of RNAs is rarely reported because these protein complexes are difficult to purify and the purified RNAPs require extended promoters and specific protein factors for transcription (Figure 7a). However, multisubunit RNAPs have potential advantages for preparative IVT: (i) high processivity, which may be essential for synthesis of longer transcripts; (ii) low transcription elongation rate, which may promote proper RNA folding; (iii) availability of an expanding collection of RNAP II *Saccharomyces cerevisiae* mutants with increased elongation rates or relaxed substrate specificity,<sup>55,56</sup> which may open new possibilities for preparative production of chemically modified transcripts. The use of yeast RNAP II for IVT is also attractive because *S. cerevisiae* is a “generally recognized as a safe” and endotoxin-free organism. Methodologies circumventing the main obstacles for IVT with multisubunit RNAPs have been developed. Purification of RNAPs was improved by addition of hexahistidine tags.<sup>57</sup> Immobilization of an RNAP on Ni-NTA affinity resin promotes its purification and allows for one-step pull down of the active RNAP from the crude cell lysate.<sup>58</sup> Furthermore, a promoter- and factor-independent system for

elongation complex assembly with core RNAP and synthetic RNA and DNA oligonucleotides was developed (Figure 7b).<sup>59</sup> This approach, combined with ligation of downstream DNA fragments to the assembled elongation complexes allows for synthesis of longer transcripts.

One important distinction of RNAP II from bacteriophage and *Escherichia coli* RNAPs is its ability to synthesize extended RNA–DNA hybrids on the ssDNA template.<sup>60</sup> We used RNAP II to synthesize RNA–DNA hybrids carrying split RNA functionalities. This is a novel promising method for functional RNA delivery.<sup>61</sup> Originally, we developed this hybrid approach (Figure 9) to separate functional nucleic acid strands and to conditionally restore their original function *in vitro* and *in vivo*.<sup>61</sup> Once inside the target cells, built-in design features (complementary ssDNA toeholds) trigger the reassociation of the hybrids and release of specific siRNAs, which effectively execute their intended therapeutic RNAi function against the target gene. We further expanded this approach to *simultaneously* deliver multiple different split functionalities for their synchronized intracellular activation (e.g., aptamers, FRET, and up to seven siRNAs at once).<sup>41</sup> Besides the tighter spatial and temporal control over synchronized activation, this novel approach may also help to resolve some problems associated with the clinical delivery of RNA-based therapies,<sup>62</sup> including intravascular degradation<sup>63</sup> (significantly reduced for RNA–DNA hybrids<sup>61</sup>) and pharmacodynamics (FRET-assisting imaging of delivery and response<sup>61</sup>). Also, additional chemical functionalities (targeting molecules or aptamers, fluorescent tags, chemical analogues of nucleotides, etc.) can be introduced through direct modifications of the DNAs in individual hybrids,

thus not interfering with the functions of the released RNA-based components.<sup>41</sup> Besides being easily produced by annealing synthetic RNAs and slightly longer DNAs (to create ssDNA toehold), the individual hybrids carrying longer RNAs (>60 nt) can be produced by RNAP II-dependent transcription of ssDNA templates.<sup>41</sup> The assembly of an elongation complex with RNAP II and a short synthetic RNA primer annealed to a ssDNA (Figure 10) followed by extension of the RNA to the



**Figure 10.** Co-transcriptional production of RNA–DNA hybrids by yeast RNAPII. (a) Hybrids with downstream DNA toeholds are obtained by stopping transcription before RNAP II runs off the template by introducing two modified nucleotides (e.g., LNAs). (b) Upstream DNA toehold containing hybrids are obtained by runoff transcription.

end of the template created the required construct with an RNA length close to 100 nt.<sup>29</sup> In the same experimental setup, *E. coli* RNAP failed to extend the RNA primer to the required length.<sup>41</sup> The T7 RNAP also appears to be less suitable for this application, because, while it transcribes partially single-stranded DNA templates,<sup>50,64</sup> production of the hybrids with the proper ssDNA toeholds was not successful.<sup>41</sup> This illustrates the importance of preparative IVT systems based on multisubunit RNAPs.

### ■ IN VIVO DELIVERY OF RNA NANOPARTICLES

One of the major hurdles in the development of RNA NPs as efficient therapeutics is their delivery *in vivo* (Figure 11). In this Account, we briefly discuss some major delivery problems and highlight approaches developed to mitigate them. There are three major obstacles for delivery of RNA NPs:

- (i) Interaction with blood components. RNA containing NPs delivered through systemic injection are confronted by the immune system to clear foreign entities circulating in the bloodstream. Naked RNAs are prone to rapid degradation by serum nucleases.<sup>52,61</sup> Serum proteins can also bind NPs inducing complement activation, inflammation, or opsonization.
- (ii) Cell targeting. The endothelium cells lining the bloodstream provide a physical filtration barrier that prevents free migration of larger particles to the tissues. Small particles can diffuse through this lining; however, NPs

smaller than 6–8 nm are also subjected to fast renal clearance.<sup>65</sup> Liver and spleen contain openings in this lining, thus allowing uptake of medium size NPs (50–100 nm), larger NPs (>200 nm) often accumulate exclusively in the spleen, and NPs with >600 nm in size accumulate in the lungs.<sup>66</sup> Due to the leaky vasculature and poor lymphatic drainage of tumors, size-dependent passive targeting can be achieved through an enhanced permeation and retention effect (EPR).<sup>67</sup>

- (iii) Cell penetration. All NPs that reached the target cells still have to cross the membrane that shields the cellular content from the extracellular milieu. This barrier is highly hydrophobic and impermeable to the negatively charged hydrophilic RNAs.

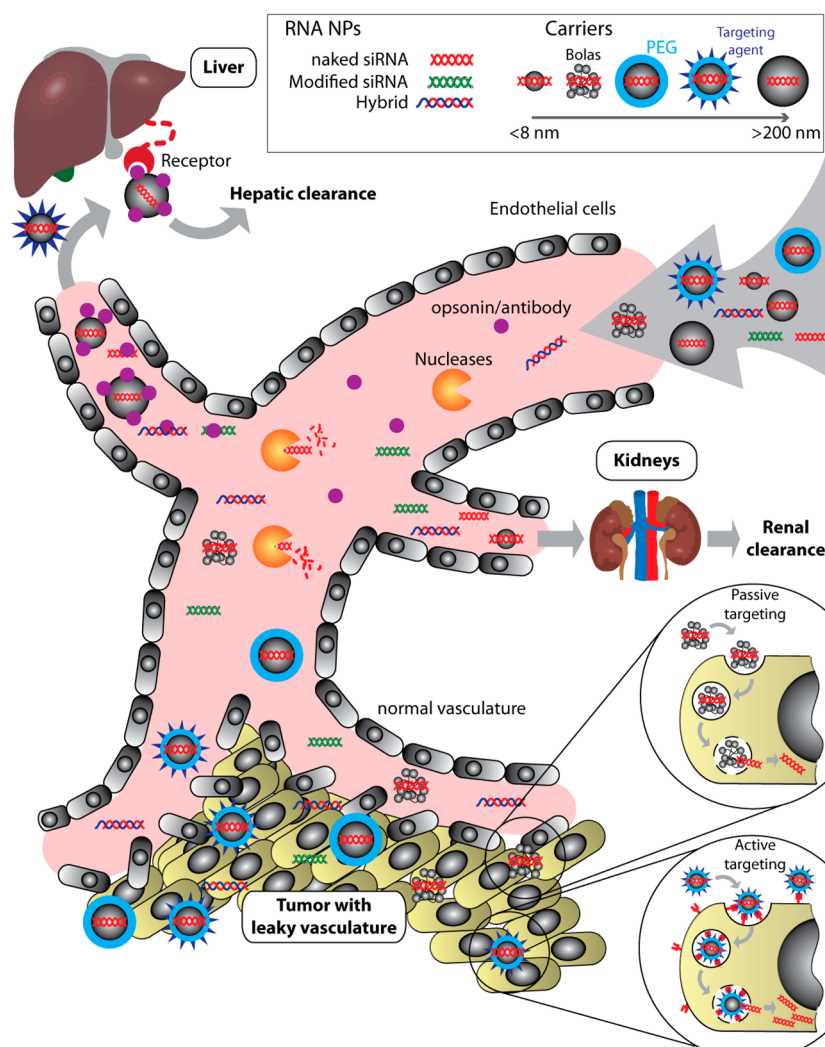
To mitigate these major obstacles, local delivery strategies are being explored<sup>68</sup> along with improvements for systemic delivery. While naked RNAs can be degraded by nucleases within minutes, proper chemical modifications can significantly improve the half-life of RNA NPs in blood.<sup>52</sup> A caveat of these modifications is the possible alteration of RNA NPs' potency and specificity.<sup>69</sup> The use of recently developed technology based on RNA–DNA hybrids improves NP stability without direct interference with RNA functionality.<sup>41,61</sup> Alternatively, to alleviate the requirement for extensive chemical modification and to improve cellular uptake of the RNA NP, protection can be achieved through the use of different carriers. A majority of these carriers are positively charged to promote a strong electrostatic interaction with RNAs. The positive charge, however, can mediate toxicity through interactions with blood components and cell membranes.<sup>70</sup> PEGylation is the widely used technique aiming to stealth-coat NPs and prolong their circulation time.<sup>71</sup> Interestingly, bolaamphiphiles (Figure 12) were recently shown to mitigate many of the obstacles on their own as a carrier.<sup>72</sup> Bolaamphiphiles' complexation with RNAs yields NPs of sizes adequate for systemic delivery while providing protection from nucleases and good transfection efficiency. Our solvent molecular dynamics simulations showed that bolaamphiphiles form stable complexes with RNAs due to electrostatic and hydrophobic interactions and hydrogen bonding. *In silico* studies were supported by various experimental studies *in vitro*, in cells, and *in vivo* using athymic nude mice bearing xenograft tumors.<sup>72</sup> Additionally, bolaamphiphile/RNA complexes do not require PEGylation, can achieve delivery through the blood–brain barrier, and despite their positive charge, show very little toxicity.

While most of the targeting is achieved passively through size and structure constraints, tissue penetration and cellular uptake can be facilitated by the presence of ligands that can confer selectivity to a particular marker or simply promote the endosomal uptake of the particles. These targeting agents (e.g., aptamers<sup>73</sup>) can be directly conjugated to the RNA NPs<sup>74</sup> or to the DNA parts of RNA–DNA hybrids.<sup>41</sup>

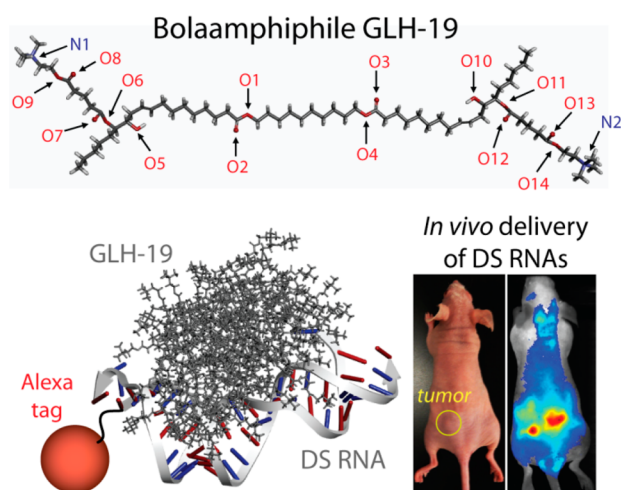
Besides the already known approaches for delivery, a promising avenue relies on the further understanding and use of delivery vehicles already present in the blood system. For example, exosomes that facilitate the transfer of genetic material could be exploited for efficient delivery of therapeutic RNA NPs.<sup>75</sup>

### ■ CONCLUSION

The study of RNA has become one of the most prominent areas in modern biology and biomedicine. By using RNA



**Figure 11.** Scheme of *in vivo* systemic delivery representing the major hurdles and the engineering solutions developed to alleviate them.



**Figure 12.** Bolaamphiphiles used for delivery of RNA NPs *in vivo*. 3D model (upper panel) and snapshot of the bolaamphiphile/RNA complex taken from MD trajectory at 50 ns (lower left panel). *In vivo* live fluorescence imaging shows accumulation of bolaamphiphile/RNA-IRDye700 complexes in the left flank tumor after tail vein injection (lower right panel). Adapted in part with permission from ref 72. Copyright 2013 Nature Publishing Group.

strands as modular scaffold units, one can engineer synthetic pathways that mimic the orchestration of native regulatory biochemical processes. It is evident that to further advance the highly promising field of RNA nanobiology, greater emphasis must be placed on basic RNA research to aim at understanding RNA structure–function relationships and RNA interactions with other classes of biological molecules such as proteins and lipids.

#### ■ ASSOCIATED CONTENT

##### Supporting Information

Details on NanoTiler and RNA2D3D. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

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##### Notes

The authors declare no competing financial interest.

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**Wojciech Kasprzak** is a Bioinformatics Analyst with Leidos Biomedical Research, Inc., working in support of Dr. Shapiro's group, NCI. Wojciech received his M.S. in Computer Science from Johns Hopkins University. His job focuses on RNA structure modeling, structure dynamics characterization, and software tools development.

**Eckart Bindewald** is a Senior Computational Scientist in Dr. Shapiro's group, NCI. Prior to that, Eckart worked on computational protein-ligand docking with Prof. Skolnick at the Donald Danforth Plant Science Center and the University at Buffalo. He earned his Ph.D. in physics from the University of Heidelberg for protein structure prediction. His current research focuses on computational RNA structure prediction and design.

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