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Synthetic RNA Scaffolds for Spatial Engineering in Cells

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13.1 Introduction

The ability to engineer cells with subcellular spatial precision is a very powerful and essential tool in synthetic biology. Specifically, co-localization of proteins, DNA, and RNA enhances metabolic output of enzymes [1, 2], allows novel regulation of gene expression [3–5], and can increase the specificity of therapeutics [6, 7]. This occurs primarily because co-localized macromolecules have high local concentrations, allowing their activities to be coordinated. Thus, better ability to organize proteins, RNAs, lipids, etc. into synthetic macromolecular complexes should enable diverse and more complex function than can be achieved by solely engineering individual parts.

In this chapter, we illustrate how synthetic RNA constructs are advancing efforts toward *in vivo* spatial engineering. Natural noncoding RNAs already play structural and catalytic roles in cells. A breadth of studies has established design principles that can be used to predictably shape RNA secondary structures [8–11]. Structural malleability of RNA, the ease of expressing synthetic RNA constructs in cells, their stability, and advances in methods for assaying and imaging assembled structures are some of the many reasons why RNA is a useful scaffolding material. Synthetic biology efforts have demonstrated that carefully designed RNA can be used for subcellular targeting of probes, enzymes, and therapeutic agents.

13.2 Structural Roles of Natural RNA

RNAs perform numerous biological functions as canonical gene expression agents, catalysts, gene regulation switches, and structural scaffolds. These struc-

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tural and catalytic roles of RNA are due in large part to the tremendous diversity of secondary and tertiary structures assumed by natural RNA and the fact that ribose sugars are more reactive than deoxyribose. RNA secondary structures can include intricate motifs like double helices, hairpin loops, bulges, pseudoknots, and right-angled turns [12, 13]. Aside from the Watson–Crick base pairing, RNA has the capacity to form Hoogsteen base pairs as well as wobble base pairs. Such interactions allow motifs to be connected in higher-order tertiary interactions, predominantly through the non-Watson–Crick base pairs [14, 15].

13.2.1 RNA as a Natural Catalyst

Catalytic roles of RNA during translation, like the tRNA shown in Figure 13.1a, disrupted a simple view held by the central dogma that RNA exists merely to transfer genetic information from DNA to protein. Today we know that RNA has catalytic and regulatory roles in many other cellular processes as well. Regulatory RNA structures play a significant role in the control of translation initiation of several bacterial genes and in bacterial immunity [17]. RNAs affect expression in *cis*, by forming secondary structures near translation start sites of the mRNA. The *cis* regions can bind to regulatory proteins or other RNAs that affect translation in *trans* [17]. Other similarly dynamic regulatory RNA regions can consist of aptamers, which are nucleic acids that selectively bind ligands

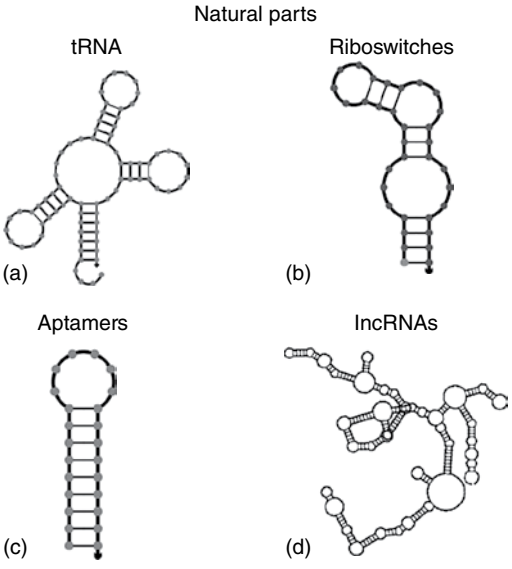

Figure 13.1 Prevalence and diversity of secondary structure in natural RNA. (a) The alanine-carrying transfer RNA shown here has the typical clover leaf structure common among tRNA. (b) The theophylline-binding riboswitch (from PDB: 1O15_A) is a canonical riboswitch. (c) The PP7 aptamer [16] binds to the PP7 coat protein with low nanomolar affinity. (d) The *Homo sapiens* TERC lncRNA (NR_001566.1) is an example of a natural lncRNA that serves as a scaffold.

[18]. Many metabolic genes are "switched" on or off, triggered by the binding of small molecule metabolites to some of these regulatory RNAs known as riboswitches (Figure 13.1b) [19].

13.2.2 RNA Scaffolds in Nature

There are also several instances of natural RNAs that are largely structural. Some natural RNAs are known to specifically bind the coat proteins of single-strand RNA phages. Such interactions help package the RNA into viral capsids. Some RNA phages that have well-characterized RNA-binding proteins include PP7 (Figure 13.1c) [16], MS2 [20], and Qβ [21]. These coat proteins also act as repressors of the viral replicase translation by specifically binding RNA hairpins near the origin of replication. In the bacteriophage Φ29, a short (117–174 nt) sequence of packaging RNA (pRNA) helps to pack phage DNA into preformed capsids [22]. A DNA packaging motor is composed of a pentameric ring of pRNA, capsid proteins, dsDNA, and an ATPase [23]. Studies characterizing the specificity and stoichiometries of these interactions [16, 24–26] have laid the foundation for RNA-tagging-based applications that we look at in Section 13.4.

RNA scaffolds are important in eukaryotic gene expression as well. Mammalian cells appear to extensively employ long noncoding RNAs (lncRNAs). These lncRNAs (Figure 13.1d) are rich with secondary structure motifs [27, 28], some of which bind and coordinate proteins on scaffolds that play important roles in epigenetic regulation [29, 30] and telomere maintenance [31, 32].

Thus, natural RNA diversity offers a template of diverse structure and function for synthetic biologists. In the following section, we look at how natural observations have been translated into an understanding of the means to precisely engineer structure and dynamics of RNA.

13.3 Design Principles for RNA Are Well Understood

In order to design, build, and test structures at the molecular scale, one must understand the physical properties of the building material. In particular, if one uses a biopolymer such as a protein or nucleic acid to build a higher-order structure, the folding properties of that polymer will dictate the structure. This is especially a challenge in the case of protein engineering, where protein structure is extremely difficult to predict *ab initio* [33, 34]. As a result, many protein engineers have focused on substituting functional rather than structural residues in existing proteins [35]. Unlike proteins, nucleic acids have a well-defined helical structure governed by a simple set of complementarity rules [36] with some exceptions such as wobble pairing and G quadruplexes [37, 38]. As a result, the structural and folding properties of RNA are generally well understood. In addition, RNA is a dynamic molecule [39–42] that can self-assemble into structures *in vitro* [13, 43–46] and can be easily transcribed from a DNA template *in vivo*. RNA functionality can also be improved using *in vitro* selection [47, 48]. For these reasons, RNA makes a suitable material for constructing synthetic *in vivo* nanostructures.

13.3.1 RNA Secondary Structure is Predictable

Most RNAs fold into a secondary structure consisting of a series of base-paired stems and unpaired loops. This secondary structure is largely determined by complementary bases within the primary RNA sequence. As a result, RNA secondary structure can be predicted computationally using a variety of methods. This typically involves using a model of the free energy of RNA base pairing [49, 50] to determine the minimum free energy secondary structure [8–11]. Structures with near-optimal folds are also calculated by these software packages, since they may be of interest, and partition functions are used to determine the relative probabilities of particular secondary structures based on their energetics (Figure 13.2a) [8–11]. Additional factors, such as wobble base pairing, pseudoknots, and dangling bases, are often incorporated into these calculations [8, 55].

Several software packages have been developed for the purpose of calculating DNA or RNA secondary structure. These include UNAFold, RNAstructure, NUPACK, and ViennaRNA [8–10, 55]. The software is typically implemented as a web server that can be used to run calculations using an online interface; it is also possible to install a local copy of the software. Each package has a somewhat different feature set (see Table 13.1 for details). For example, RNAstructure can

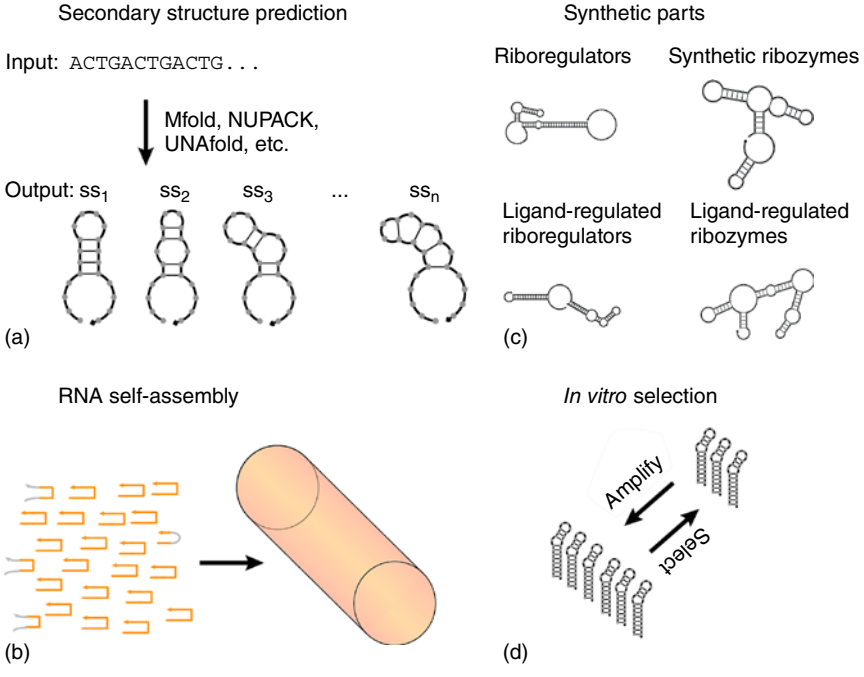

Figure 13.2 Design principles for RNA structure and function. (a) RNA secondary structure can be predicted from the primary sequence using a variety of software packages. (b) RNA can self-assemble into 2D or 3D structures *in vitro*. (c) Researchers have developed a variety of synthetic parts, such as synthetic riboregulators, synthetic ribozymes, ligand-regulated riboregulators, and ligand-regulated ribozymes [51–54]. (d) *In vitro* selection can be used to enhance the function of RNAs through iterative rounds of amplification and selection.

Table 13.1 Comparison of features between RNA structure prediction software packages.

Feature	NUPACK	RNAstructure	UNAfold	ViennaRNA
MFE calculation	●	●	●	●
Partition function	●	●	●	●
Wobble pairing	●	●	●	●
Pseudoknots	●	●	○	○
Dangling bases	●	●	●	●
Multi-strand interactions	●	○	○	○
Uses SHAPE/NMR data	○	●	○	○
Graphical User Interface	○	●	○	○
Web Interface	●	●	●	●

A filled-in circle indicates that the software package contains the feature in a row, whereas an empty circle indicates that the software package does not contain the feature in a row.
MFE, minimum free energy.

integrate user-supplied experimental data such as selective 2′ hydroxylation and primer extension (SHAPE) [56] or NMR to aid in structure calculation and has a convenient graphical user interface [10]. ViennaRNA is designed to be computationally efficient for testing many RNA structures in batches rather than for analyzing individual species in more detail [8]. UNAFold is derived from mfold, which used the first dynamic programming algorithm for predicting RNA secondary structure [9, 57]. A particularly useful package for designing RNA structures is NUPACK, which can handle multi-strand interactions and allows the user to design sequences that have a propensity to assemble into a user-defined set of secondary structures [55, 58]. Given the diversity of software packages for predicting RNA secondary structure, it is important to choose the right software package for one's particular design needs.

13.3.2 RNA can Self-Assemble into Structures

RNA can self-assemble into geometrically precise structures *in vitro* (Figure 13.2b). This was first shown for small RNA molecules with four stem-loops (tectoRNAs), which self-assemble into 1D structures using kissing loops [59], but has since been extended to form a variety of geometrically precise 2D and 3D shapes [13, 43–46, 60]. Of particular note are the *in vivo* RNA assemblies [1], which can self-assemble into 1D or 2D lattices. Although *in vitro* structures have traditionally been formed using a thermal annealing process, recent work has shown that single-stranded DNA tiles and bricks [61, 62] can self-assemble into discrete nanostructures isothermally and under biocompatible conditions [63]. Thus, it is possible to self-assemble a diverse range of scaffolds using RNA.

13.3.3 Dynamic RNAs can be Rationally Designed

Beyond structure formation, RNA also has the capability to dynamically reconfigure itself in response to small molecules or other ligands [39–42]. Such

RNAs – ribozymes and riboswitches, respectively – underscore the notion that RNAs can be dynamic molecules. However, RNAs can also be rationally designed to go beyond their natural function (Figure 13.2c). For example, synthetic riboregulators can be designed to control genes in the presence of a user-defined input RNA molecule [51]. It is even possible to combine pairs of functional RNAs to form more complicated devices, such as by combining riboswitches with ribozymes [64], riboswitches with riboregulators [52, 65], or aptamers with transcriptional attenuators [66]. These compound RNA devices underscore the notion that RNA secondary structure can be programmed to achieve a range of dynamic functions.

13.3.4 RNA can be Selected *in vitro* to Enhance Its Function

Another powerful technique that has aided the development of many functional RNA motifs is *in vitro* selection or systematic evolution of ligands by exponential enrichment (SELEX) [47, 48] (Figure 13.2d). This typically involves starting with a library of many (10^{13}–10^{15}) distinct RNA sequences and then applying iterative rounds of selection (e.g., binding to a small molecule immobilized on a surface or catalyzing ligation to a surface-bound ligand) and amplification (typically involving polymerase chain reaction (PCR)). After ~10 rounds of selection and amplification, the activity of the remaining RNA sequences in the pool can be enhanced by several orders of magnitude compared with the initial library average [67]. Some functions may not be present in a library of 10^{15} RNAs; thus it may sometimes be necessary to chemically modify or structurally bias the initial library [67]. This limitation aside, *in vitro* selection is a useful technique for generating synthetic RNAs with specific functions.

In the two decades since the development of *in vitro* selection, thousands of aptamers (oligonucleotides that bind to a particular ligand) have been developed [68]. These include aptamers to small molecules, peptides, and even human and cancer cell types [47, 67, 69–71]. In addition to RNA molecules, proteins such as epitopes and antibodies have been evolved using *in vitro* selection [72–74]. Thus, *in vitro* selection can be used to enhance functional portions of an RNA scaffold. This is especially useful when existing RNA parts are not sufficient for the task at hand.

13.4 Applications of Designed RNA Scaffolds

RNA sequences consisting of secondary structures and functional units designed using the tools described previously can be genetically expressed in cells. Such engineered RNAs have been used for tasks ranging from studying natural RNA processing in cells to metabolic engineering and therapeutic applications.

13.4.1 Tools for RNA Research

While mRNA has long been known to function as a template for protein translation, the spatiotemporal aspects of the various steps involved in mRNA processing

remain poorly understood. Investigation of the dynamics of mRNA as it goes through translation, splicing, nuclear export in eukaryotes, localization for translation, and finally degradation requires tools to track individual RNA molecules. Aptamers and their recruitment of fluorescent proteins on engineered mRNA scaffolds have enabled such studies.

Some of the earliest attempts to tag RNA *in vivo* were carried out by expressing GFP fused with bacteriophage MS2 coat protein [75] or human RNA-interacting protein domain U1A [76] along with mRNA containing the corresponding binding sites in *Saccharomyces cerevisiae*. Such tags enabled tracking of single-cell mRNA localization by microscopy. Furthermore, by incorporating tandem repeats of MS2 binding sites on reporter mRNA [77], several GFP–MS2 fusions could be localized on a single transcript, enabling tracking of individual mRNA molecules in mammalian cells (Figure 13.3a). This *in vivo* tracking method was extended to other systems [82], including bacteria [78, 83].

More recently, several efforts have addressed the long-standing question of whether or not RNA is highly localized within bacterial cells [84, 85]. A significant innovation over the previous strategy came from the use of fluorescent protein complementation. In this approach, RNA aptamers are used to bring together two different protein fusion units, each with a split fluorescent protein fused to an RNA-binding domain (RBD) [79, 86] (Figure 13.3a). Since only the scaffolded protein units are able to reconstitute the split chromophore and fluoresce, they can be easily distinguished from the unbound ones. Such an approach hence achieves lower background signals than systems where autofluorescent proteins are directly tagged onto RNA.

As the repertoire of aptamer–RNA-binding protein pairs is being extended through the *in vitro* methods described in Section 13.3.4, newer combinations are being used to explore cellular function [87]. The studies discussed here have led to a better understanding of RNA diffusion and localization [78, 79] in bacterial cells and measurement of transcriptional kinetics [88]. These efforts also enabled localization of a diverse array of proteins (such as enzymes) on RNA scaffolds, opening up applications in metabolic engineering.

13.4.2 Localizing Metabolic Enzymes on RNA

Scaffolding and compartmentalization are effective strategies for optimization of metabolic pathway performance in both natural and synthetic systems [89, 90]. A few studies have used DNA structures to coordinate the assembly of enzymes and study effects of spatial co-localization *in vitro* [91–94] and *in vivo* [95]. Protein scaffolds have also been used to channel metabolic substrates between co-localized enzymes in living cells [2, 96]. Scaffolding is seen as a powerful tool to specifically direct metabolic pathway flux toward enzymes of choice, prevent loss of intermediates to competing reactions, and protect the host cell from any toxic or volatile intermediates through confinement at a subcellular location.

A notable effort in the use of RNA scaffolds for metabolic channeling achieved a nearly 50-fold increase in hydrogen gas production in *Escherichia coli* [1]. This effort combined many of the techniques discussed previously. Synthetic RNA

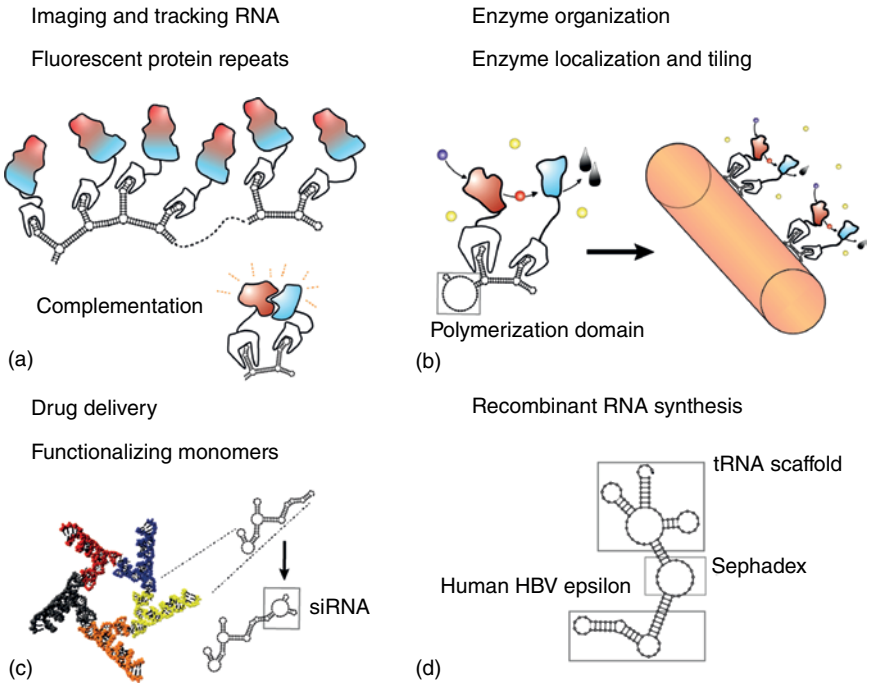

Figure 13.3 Applications of RNA scaffolds *in vivo*. (a) mRNA are modified to include either several repeats of an aptamer or two different aptamers in close proximity. The former approach results in concentrated foci of fluorescent protein fusions to RNA-binding domains (RBDs) [78] and in the latter, two halves of the protein with RBD fusions [79], only complement to be fluorescent on the mRNA scaffold. (b) Enzymes fused to RBDs localize to self-assembled RNA scaffolds with aptamers presented. Channeling of intermediate metabolites can lead to enhanced pathway flux toward biofuels or other high value products [1]. (c) Pentamer of bacteriophage Φ29 pRNA [23] from PDB file 1FOQ. Tagging the monomers with functional units like siRNA can make them useful drug delivery vehicles [6, 80]. (d) The clover leaf tRNA sequence can be tagged with recombinant RNA and epitopes as shown to allow for its synthesis and purification [81].

strands comprising polymerization domains and aptamers for MS2 and PP7 coat proteins were expressed in the bacteria. Dimerization and polymerization domains allowed for tiling and assembly into a macromolecular structure. The large (40–100 nm) intracellular RNA assemblies greatly enhanced the flux of electrons from ferredoxin to hydrogenase when both enzymes were tethered to the scaffold with fusions to MS2 and PP7 (Figure 13.3b). Furthermore, significant differences in titer were observed for scaffolding structures having different geometries, tying metabolic flux to the specific spatial positioning of the scaffold. Such an approach brings modular design and scalability [97] to metabolic engineering for biofuels and high value chemical synthesis, where control of intermediate metabolite flux can be critical [98–100].

There has been debate about the mechanism by which scaffolds enable metabolic substrate channeling. The transfer of electrons between enzymes relies on physical contact and thus is limited by protein diffusion rates and competition,

which are effectively addressed by scaffolding [1]. However, the role of enzyme co-localization in pathways involving diffusible intermediates is much less well understood [101, 102]. In a recent study [103], enzymes localized in close proximity, less than 30 nm apart, on *in vitro* assembled DNA scaffolds exhibited enhanced rates of metabolite exchange. The transfer rates dropped precipitously with any further increase in interenzyme distance. Since such effects are not explicable by 3D diffusion models [101], a mechanism of metabolite substrate channeling by restricted diffusion on hydration layers across crowded protein surfaces has been proposed [103]. RNA scaffolds, with their predictable geometry, can be used to create a range of metabolic channeling platforms and test the relative effects from these two different mechanisms.

13.4.3 Packaging Therapeutics on RNA Scaffolds

While metabolic channeling functions relied on RNA interactions with proteins, RNA–RNA interactions can also be used for exciting scaffold applications. pRNA from bacteriophage Φ29 (referred to in Section 13.2) has been used as a building block for bottom-up assembly of drug delivery vehicles [6, 80] (Figure 13.3c). pRNA monomers consist of structural hairpin regions and dimerization/polymerization domains. Ends of the hairpin regions offer sites for tagging with drugs or targeting molecules. The polymerization domains can be engineered to favor formation of dimers, trimers, pentamers, or hexamers as stable drug carriers [6, 23, 80]. Heterodimers containing pRNA tagged with a CD4 aptamer and pRNA attached to an siRNA were shown to specifically target CD4-expressing T cells, leading to cell death [80]. This *in vitro* study also showed stability and efficacy of the nanoscale drug delivery particles for killing cancer cells. Such systems are advantageous since the pRNA polymers are hypothesized to be stable in physiological conditions and be less immunogenic than protein carriers [80]. Finally, these polymers could be made specific to many *in situ* targets by using engineered specific RNA aptamers that recognize cellular moieties.

13.4.4 Recombinant RNA Technology

RNA scaffolds have also been used to serve as protective tethers for the purification of recombinant RNA (recRNA) (Figure 13.3d) [81]. In this approach, a tRNA scaffold acts as a protective secondary structure to insulate the transcript from native *E. coli* nucleases and therefore stabilize production of recRNA *in vivo*. The characteristic clover leaf tRNA structure formed around a recRNA is recognized by native cellular enzymes and processed as tRNA. This ensures that each single transcript is a product of specific defined length. A Sephadex affinity tag was included in the expressed sequence to allow purification of transcripts that contained RNAs of medical research interest, like the human hepatitis B virus (HBV) epsilon [81]. This design thus enables collection of large amounts of purified RNA transcripts for *in vitro* structural studies and vaccine development. Recently, these efforts have been extended to expression and purification of RNA–protein complexes [104], providing pure samples that could be used for crystallographic studies of natural RNA–protein interactions and potential use in cell-free systems.

13.5 Conclusion

RNA is a powerful tool to synthetic biologists. RNA scaffolds can be composed of many structural, dynamic, and functional regions. Structure design can be predicted reliably, and there are a growing number of assays for proper structure assembly. In addition, recent advances in DNA construction [105, 106] have made it faster and easier to test new structure designs *in vivo*. Prediction and design of RNA structure in three dimensions remains a challenge. The difficulty of going from a secondary structure design to precise orientation of tertiary scaffold units needs to be addressed for metabolic engineering and therapeutic applications. Additionally, although localization of fluorophores to RNA enables *in vivo* imaging, resolution limits have prevented elucidation of precise geometric details in RNA scaffolds and assemblies within cells. Future technical advances could enable many scientists to construct new RNA scaffolds for a wide range of purposes. In the following text, we discuss a particular set of exciting applications and the technologies that will enable them.

13.5.1 New Applications

Synthetic biologists are constantly seeking to increase the complexity of their devices. RNA synthetic biology is offering tools to enable such control [107]. One particular goal is the construction of orthogonal ribosomes [108], capable of incorporating nonnatural amino acids wherein altered tRNA–protein interactions enable an expanded genetic code [109]. RNA scaffolds are also being employed to devise more precise genome editing tools [110]. For metabolic engineering applications, RNA scaffolds are enabling control over the relative geometric orientations of enzymes in a co-localized pathway, which can lead to better channeling of volatile intermediate metabolites [111]. Therapeutic applications of *in vivo* RNA scaffolds include functionalizing natural RNA scaffolds to enable drug delivery or isolation of pure samples. Similar developments in the fields of DNA packaging and origami for drug delivery [112, 113] could offer strong synergistic opportunities for clinically applicable technologies to be implemented. More generally, the ability to simulate and predict the dynamics of structure-receptor binding interactions should enhance the design of such therapeutics [114].

13.5.2 Technological Advances

Moving forward, innovations in high-throughput design, synthesis, and assaying functions for RNA structures will enable a greater range of applications to be developed. *In silico* design software packages are continuously improving their capabilities, making it possible to computationally generate increasingly complicated structures [55]. In addition to the advances for *in vivo* synthesis and purification of RNAs mentioned previously, developments in chip-based synthesis could enable hundreds of RNA designs to be synthesized *in vitro* at a time [106, 115]. This, coupled with new structure assembly assays such as SHAPE-Seq [116] and improved genetically encodable electron microscopy tags [117, 118], will greatly simplify the testing of more complicated structures. Developments in

RNA imaging [119] can be further advanced by incorporation of docking sites that allow RNA to be probed with oligonucleotides using methods like DNA-PAINT [120], leading to super-resolution imaging *in situ*.

Thus, the discovery of a variety of natural RNA structures and functions, an ever-increasing understanding of how such features can be designed, and an ability to rapidly implement and test ideas are indicators of a significant role for RNA scaffolds in future synthetic biology applications.

Definitions

Synthetic biology is a discipline that seeks to control biology using the principles of engineering

Nanotechnology is the manipulation of matter at the atomic, molecular, and supramolecular scale

RNA scaffolds are macromolecular structures or assemblies of RNA with well-defined secondary structure motifs for spatially organizing other biomolecules. These are typically expressed in living cells for metabolic engineering purposes

Isothermal assembly is a self-assembly of structures at a constant temperature

Metabolic engineering is the production of small molecules or short peptides through the engineering of metabolic pathways

Aptamers are nucleic acid oligonucleotides that bind a specific small molecule or other ligand

References

1 Delebecque, C.J., Lindner, A.B., Silver, P.A., and Aldaye, F.A. (2011) Organization of intracellular reactions with rationally designed RNA assemblies. *Science*, **333** (6041), 470–474.

2 Dueber, J.E., Wu, G.C., Malmirchegini, G.R., Moon, T.S., Petzold, C.J., Ullal, A.V., Prather, K.L.J., and Keasling, J.D. (2009) Synthetic protein scaffolds provide modular control over metabolic flux. *Nat. Biotechnol.*, **27** (8), 753–759.

3 Isaacs, F.J., Dwyer, D.J., and Collins, J.J. (2006) RNA synthetic biology. *Nat. Biotechnol.*, **24** (5), 545–554.

4 Culler, S.J., Hoff, K.G., and Smolke, C.D. (2010) Reprogramming cellular behavior with RNA controllers responsive to endogenous proteins. *Science*, **330** (6008), 1251–1255.

5 Qi, L.S., Larson, M.H., Gilbert, L.A., Doudna, J.A., Weissman, J.S., Arkin, A.P., and Lim, W.A. (2013) Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell*, **152** (5), 1173–1183.

6 Khaled, A., Guo, S., Li, F., and Guo, P. (2005) Controllable self-assembly of nanoparticles for specific delivery of multiple therapeutic molecules to cancer cells using RNA nanotechnology. *Nano Lett.*, **5** (9), 1797–1808.

7 Aldaye, F.A., Senapedis, W.T., Silver, P.A., and Way, J.C. (2010) A structurally tunable DNA-based extracellular matrix. *J. Am. Chem. Soc.*, **132** (42), 14727–14729.

8 Lorenz, R., Bernhart, S.H., Siederdissen, C.H.Z., Tafer, H., Flamm, C., Stadler, P.F., and Hofacker, I.L. (2011) ViennaRNA Package 2.0. *Algorithms Mol. Biol.*, **6** (1), 26.

9 Markham, N.R. and Zuker, M. (2008) UNAFold: software for nucleic acid folding and hybridization. *Methods Mol. Biol.*, **453**, 3–31.

10 Reuter, J.S. and Mathews, D.H. (2010) RNA structure: software for RNA secondary structure prediction and analysis. *BMC Bioinf.*, **11** (1), 129.

11 Zadeh, J.N., Steenberg, C.D., Bois, J.S., Wolfe, B.R., Pierce, M.B., Khan, A.R., Dirks, R.M., and Pierce, N.A. (2010) NUPACK: analysis and design of nucleic acid systems. *J. Comput. Chem.*, **32** (1), 170–173.

12 Leontis, N.B., Lescoute, A., and Westhof, E. (2006) The building blocks and motifs of RNA architecture. *Curr. Opin. Struct. Biol.*, **16** (3), 279–287.

13 Jaeger, L. and Chworos, A. (2006) The architectonics of programmable RNA and DNA nanostructures. *Curr. Opin. Struct. Biol.*, **16** (4), 531–543.

14 Cruz, J.A. and Westhof, E. (2009) The dynamic landscapes of RNA architecture. *Cell*, **136** (4), 604–609.

15 Tinoco, I. Jr. and Bustamante, C. (1999) How RNA folds. *J. Mol. Biol.*, **293** (2), 271–281.

16 Lim, F.F., Downey, T.P.T., and Peabody, D.S.D. (2001) Translational repression and specific RNA binding by the coat protein of the Pseudomonas phage PP7. *J. Biol. Chem.*, **276** (25), 22507–22513.

17 Waters, L.S. and Storz, G. (2009) Regulatory RNAs in bacteria. *Cell*, **136** (4), 615–628.

18 Winkler, W.C. and Breaker, R.R. (2005) Regulation of bacterial gene expression by riboswitches. *Annu. Rev. Microbiol.*, **59**, 487–517.

19 Nudler, E. and Mironov, A.S. (2004) The riboswitch control of bacterial metabolism. *Trends Biochem. Sci*, **29** (1), 11–17.

20 Hirao, I., Spingola, M., Peabody, D., and Ellington, A.D. (1998) The limits of specificity: an experimental analysis with RNA aptamers to MS2 coat protein variants. *Mol. Diversity*, **4** (2), 75–89.

21 Witherell, G.W. and Uhlenbeck, O.C. (1989) Specific RNA binding by Q.beta. coat protein. *Biochemistry*, **28** (1), 71–76.

22 Guo, P., Erickson, S., and Anderson, D. (1987) A small viral RNA is required for in vitro packaging of bacteriophage phi 29 DNA. *Science*, **236** (4802), 690–694.

23 Simpson, A.A., Tao, Y., Leiman, P.G., Badasso, M.O., He, Y., Jardine, P.J., Olson, N.H., Morais, M.C., Grimes, S., Anderson, D.L., Baker, T.S., and Rossmann, M.G. (2000) Structure of the bacteriophage phi29 DNA packaging motor. *Nature*, **408** (6813), 745–750.

24 Ni, C.-Z., Syed, R., Kodandapani, R., Wickersham, J., Peabody, D.S., and Ely, K.R. (1995) Crystal structure of the MS2 coat protein dimer: implications for RNA binding and virus assembly. *Structure*, **3** (3), 255–263.

25 Peabody, D.S. and Ely, K.R. (1992) Control of translational repression by protein–protein interactions. *Nucleic Acids Res.*, **20** (7), 1649–1655.

26 Guo, P., Zhang, C., Chen, C., Garver, K., and Trottier, M. (1998) Inter-RNA interaction of phage ϕ29 pRNA to form a hexameric complex for viral DNA transportation. *Mol. Cell*, **2** (1), 149–155.

27 Underwood, J.G., Uzilov, A.V., Katzman, S., Onodera, C.S., Mainzer, J.E., Mathews, D.H., Lowe, T.M., Salama, S.R., and Haussler, D. (2010) FragSeq:

transcriptome-wide RNA structure probing using high-throughput sequencing. *Nat. Methods*, **7** (12), 995–1001.

28 Kertesz, M., Wan, Y., Mazor, E., Rinn, J.L., Nutter, R.C., Chang, H.Y., and Segal, E. (2010) Genome-wide measurement of RNA secondary structure in yeast. *Nature*, **467** (7311), 103–107.

29 Mercer, T.R. and Mattick, J.S. (2013) Structure and function of long noncoding RNAs in epigenetic regulation. *Nat. Struct. Mol. Biol.*, **20** (3), 300–307.

30 Tsai, M.C., Manor, O., Wan, Y., Mosammaparast, N., Wang, J.K., Lan, F., Shi, Y., Segal, E., and Chang, H.Y. (2010) Long noncoding RNA as modular scaffold of histone modification complexes. *Science*, **329** (5992), 689–693.

31 Zappulla, D.C. and Cech, T.R. (2004) Yeast telomerase RNA: a flexible scaffold for protein subunits. *Proc. Natl. Acad. Sci. U.S.A.*, **101** (27), 10024–10029.

32 Theimer, C.A. and Feigon, J. (2006) Structure and function of telomerase RNA. *Curr. Opin. Struct. Biol.*, **16** (3), 307–318.

33 Arnold, F.H. (2001) Combinatorial and computational challenges for biocatalyst design. *Nature*, **409** (6817), 253–257.

34 Bonneau, R. and Baker, D. (2001) Ab initio protein structure prediction: progress and prospects. *Annu. Rev. Biophys. Biomol. Struct.*, **30**, 173–189.

35 Bornscheuer, U.T., Huisman, G.W., Kazlauskas, R.J., Lutz, S., Moore, J.C., and Robins, K. (2012) Engineering the third wave of biocatalysis. *Nature*, **485** (7397), 185–194.

36 Watson, J.D. and Crick, F.H. (1953) Molecular structure of nucleic acids. *Nature*, **171** (4356), 737–738.

37 Varani, G. and McClain, W.H. (2000) The G· U wobble base pair. *EMBO Rep.*, **1** (1), 18–23.

38 Lipps, H.J. and Rhodes, D. (2009) G-quadruplex structures: in vivo evidence and function. *Trends Cell Biol.*, **19** (8), 414–422.

39 Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N., and Altman, S. (1983) The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. *Cell*, **35** (3), 849–857.

40 Kruger, K., Grabowski, P.J., Zaug, A.J., Sands, J., Gottschling, D.E., and Cech, T.R. (1982) Self-splicing RNA: autoexcision and autocyclization of the ribosomal RNA intervening sequence of *Tetrahymena*. *Cell*, **31** (1), 147–157.

41 Nahvi, A., Sudarsan, N., Ebert, M.S., Zou, X., Brown, K.L., and Breaker, R.R. (2002) Genetic control by a metabolite binding mRNA. *Chem. Biol.*, **9** (9), 1043.

42 Winkler, W., Nahvi, A., and Breaker, R.R. (2002) Thiamine derivatives bind messenger RNAs directly to regulate bacterial gene expression. *Nature*, **419** (6910), 952–956.

43 Afonin, K.A., Bindewald, E., Yaghoubian, A.J., Voss, N., Jacovetty, E., Shapiro, B.A., and Jaeger, L. (2010) In vitro assembly of cubic RNA-based scaffolds designed in silico. *Nat. Nanotechnol.*, **5** (9), 676–682.

44 Chworos, A., Severcan, I., Koyfman, A.Y., Weinkam, P., Oroudjev, E., Hansma, H.G., and Jaeger, L. (2004) Building programmable Jigsaw puzzles with RNA. *Science*, **306** (5704), 2068–2072.

45 Dibrov, S.M., McLean, J., Parsons, J., and Hermann, T. (2011) Self-assembling RNA square. *Proc. Natl. Acad. Sci. U.S.A.*, **108** (16), 6405–6408.

46 Severcan, I., Geary, C., Chworos, A., Voss, N., Jacovetty, E., and Jaeger, L. (2010) A polyhedron made of tRNAs. *Nat. Chem.*, **2** (9), 772–779.

47 Ellington, A.D. and Szostak, J.W. (1990) In vitro selection of RNA molecules that bind specific ligands. *Nature*, **346** (6287), 818–822.

48 Tuerk, C. and Gold, L. (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science*, **249** (4968), 505–510.

49 Walter, A.E., Turner, D.H., Kim, J., Lyttle, M.H., Müller, P., Mathews, D.H., and Zuker, M. (1994) Coaxial stacking of helixes enhances binding of oligoribonucleotides and improves predictions of RNA folding. *Proc. Natl. Acad. Sci. U.S.A.*, **91** (20), 9218–9222.

50 Mathews, D.H., Sabina, J., Zuker, M., and Turner, D.H. (1999) Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *J. Mol. Biol.*, **288** (5), 911–940.

51 Isaacs, F.J., Dwyer, D.J., Ding, C., Pervouchine, D.D., Cantor, C.R., and Collins, J.J. (2004) Engineered riboregulators enable post-transcriptional control of gene expression. *Nat. Biotechnol.*, **22** (7), 841–847.

52 Bayer, T.S. and Smolke, C.D. (2005) Programmable ligand-controlled riboregulators of eukaryotic gene expression. *Nat. Biotechnol.*, **23** (3), 337–343.

53 Lou, C., Stanton, B., Chen, Y.-J., Munsky, B., and Voigt, C.A. (2012) Ribozyme-based insulator parts buffer synthetic circuits from genetic context. *Nat. Biotechnol.*, **30** (11), 1137–1142.

54 Win, M.N. and Smolke, C.D. (2007) Targeted cleavage: tuneable cis-cleaving ribozymes. *Proc. Natl. Acad. Sci. U.S.A.*, **104** (38), 14881–14882.

55 Zadeh, J.N., Wolfe, B.R., and Pierce, N.A. (2010) Nucleic acid sequence design via efficient ensemble defect optimization. *J. Comput. Chem.*, **32** (3), 439–452.

56 Merino, E.J., Wilkinson, K.A., Coughlan, J.L., and Weeks, K.M. (2005) RNA structure analysis at single nucleotide resolution by selective 2′-hydroxyl acylation and primer extension (SHAPE). *J. Am. Chem. Soc.*, **127** (12), 4223–4231.

57 Zuker, P.S.M. (1981) Optimal computer folding of large RNA sequences using thermodynamics and auxiliary information. *Nucleic Acids Res.*, **9** (1), 133.

58 Dirks, R.M., Bois, J.S., Schaeffer, J.M., Winfree, E., and Pierce, N.A. (2007) Thermodynamic analysis of interacting nucleic acid strands. *SIAM Rev.*, **49** (1), 65–88.

59 Jaeger, L. and Leontis, N. (2000) Tecto-RNA: one-dimensional self-assembly through tertiary interactions *Angew. Chem. Int. Ed.*, **39** (14), 2521–2524.

60 Khisamutdinov, E.F., Jasinski, D.L., and Guo, P. (2014) RNA as a boiling-resistant anionic polymer material to build robust structures with defined shape and stoichiometry. *ACS Nano*, **8** (5), 4771–4781.

61 Wei, B., Dai, M., and Yin, P. (2012) Complex shapes self-assembled from single-stranded DNA tiles. *Nature*, **485** (7400), 623–626.

62 Ke, Y., Ong, L.L., Shih, W.M., and Yin, P. (2012) Three-dimensional structures self-assembled from DNA bricks. *Science*, **338** (6111), 1177–1183.

63 Myhrvold, C., Dai, M., Silver, P.A., and Yin, P. (2013) Isothermal self-assembly of complex DNA structures under diverse and biocompatible conditions. *Nano Lett.*, **13** (9), 4242–4248.

64 Win, M.N. and Smolke, C.D. (2007) A modular and extensible RNA-based gene-regulatory platform for engineering cellular function. *Proc. Natl. Acad. Sci. U.S.A.*, **104** (36), 14283–14288.

65 Beisel, C.L., Bayer, T.S., Hoff, K.G., and Smolke, C.D. (2008) Model-guided design of ligand-regulated RNAi for programmable control of gene expression. *Mol. Syst. Biol.*, **4** (224).

66 Qi, L., Lucks, J.B., Liu, C.C., Mutalik, V.K., and Arkin, A.P. (2012) Engineering naturally occurring trans-acting non-coding RNAs to sense molecular signals. *Nucleic Acids Res.*, **40** (12), 5775–5786.

67 Wilson, D.S. and Szostak, J.W. (1999) In vitro selection of functional nucleic acids. *Annu. Rev. Biochem.*, **68** (1), 611–647.

68 Lee, J.F., Hesselberth, J.R., Meyers, L.A., and Ellington, A.D. (2004) Aptamer database. *Nucleic Acids Res.*, **32** (Database issue), D95–D100.

69 Davis, J.H. and Szostak, J.W. (2002) Isolation of high-affinity GTP aptamers from partially structured RNA libraries. *Proc. Natl. Acad. Sci. U.S.A.*, **99** (18), 11616–11621.

70 Colas, P., Cohen, B., Jessen, T., Grishina, I., McCoy, J., and Brent, R. (1996) Genetic selection of peptide aptamers that recognize and inhibit cyclin-dependent kinase 2. *Nature*, **380** (6574), 548–550.

71 Shangguan, D., Li, Y., Tang, Z., Cao, Z.C., Chen, H.W., Mallikaratchy, P., Sefah, K., Yang, C.J., and Tan, W. (2006) Aptamers evolved from live cells as effective molecular probes for cancer study. *Proc. Natl. Acad. Sci. U.S.A.*, **103** (32), 11838–11843.

72 Hanes, J. and Plückthun, A. (1997) In vitro selection and evolution of functional proteins by using ribosome display. *Proc. Natl. Acad. Sci. U.S.A.*, **94** (10), 4937–4942.

73 Roberts, R.W. and Szostak, J.W. (1997) RNA-peptide fusions for the in vitro selection of peptides and proteins. *Proc. Natl. Acad. Sci. U.S.A.*, **94** (23), 12297–12302.

74 Bayer, T.S., Booth, L.N., Knudsen, S.M., and Ellington, A.D. (2005) Arginine-rich motifs present multiple interfaces for specific binding by RNA. *RNA*, **11** (12), 1848–1857.

75 Bertrand, E., Chartrand, P., Schaefer, M., Shenoy, S.M., Singer, R.H., and Long, R.M. (1998) Localization of ASH1 mRNA particles in living yeast. *Mol. Cell*, **2** (4), 437–445.

76 Brodsky, A.S. and Silver, P.A. (2000) Pre-mRNA processing factors are required for nuclear export. *RNA*, **6** (12), 1737–1749.

77 Fusco, D., Accornero, N., Lavoie, B., Shenoy, S.M., Blanchard, J.-M., Singer, R.H., and Bertrand, E. (2003) Single mRNA molecules demonstrate probabilistic movement in living mammalian cells. *Curr. Biol.*, **13** (2), 161–167.

78 Golding, I. and Cox, E.C. (2004) RNA dynamics in live *Escherichia coli* cells. *Proc. Natl. Acad. Sci. U.S.A.*, **101** (31), 11310–11315.

79 Valencia-Burton, M., McCullough, R.M., Cantor, C.R., and Broude, N.E. (2007) RNA visualization in live bacterial cells using fluorescent protein complementation. *Nat. Methods*, **282** (5387), 296–298.

80 Guo, S., Tschammer, N., Mohammed, S., and Guo, P. (2005) Specific delivery of therapeutic RNAs to cancer cells via the dimerization mechanism of phi29 motor pRNA. *Hum. Gene Ther.*, **16** (9), 1097–1109.

81 Ponchon, L. and Dardel, F. (2007) Recombinant RNA technology: the tRNA scaffold. *Nat. Methods*, **4** (7), 571–576.

82 Schifferer, M. and Griesbeck, O. (2009) Application of aptamers and autofluorescent proteins for RNA visualization. *Integr. Biol.*, **1** (8), 499–505.

83 Le, T.T., Harlepp, S., Guet, C.C., Dittmar, K., Emonet, T., Pan, T., and Cluzel, P. (2005) Real-time RNA profiling within a single bacterium. *Proc. Natl. Acad. Sci. U.S.A.*, **102** (2), 9160–9164.

84 Keiler, K.C. (2011) RNA localization in bacteria. *Curr. Opin. Microbiol.*, **14** (2), 155–159.

85 Broude, N.E. (2011) Analysis of RNA localization and metabolism in single live bacterial cells: achievements and challenges. *Mol. Microbiol.*, **80** (5), 1137–1147.

86 Ozawa, T., Natori, Y., Sato, M., and Umezawa, Y. (2007) Imaging dynamics of endogenous mitochondrial RNA in single living cells. *Nat. Methods*, **4** (5), 413–419.

87 Yiu, H.-W., Demidov, V.V., Toran, P., Cantor, C.R., and Broude, N.E. (2011) RNA detection in live bacterial cells using fluorescent protein complementation triggered by interaction of two RNA aptamers with two RNA-binding peptides. *Pharmaceuticals*, **4** (3), 494–508.

88 Valencia-Burton, M., Shah, A., Sutin, J., Borogovac, A., McCullough, R.M., Cantor, C.R., Meller, A., and Broude, N.E. (2009) Spatiotemporal patterns and transcription kinetics of induced RNA in single bacterial cells. *Proc. Natl. Acad. Sci. U.S.A.*, **106** (38), 16399–16404.

89 Agapakis, C.M., Boyle, P.M., and Silver, P.A. (2012) Natural strategies for the spatial optimization of metabolism in synthetic biology. *Nat. Chem. Biol.*, **8** (6), 527–535.

90 Chen, A.H. and Silver, P.A. (2012) Designing biological compartmentalization. *Trends Cell Biol.*, **22** (12), 662–670.

91 Erkelenz, M., Kuo, C.-H., and Niemeyer, C.M. (2011) DNA-mediated assembly of cytochrome P450 BM3 subdomains. *J. Am. Chem. Soc.*, **133** (40), 16111–16118.

92 Liu, M., Fu, J., Hejesen, C., Yang, Y., Woodbury, N.W., Gothelf, K., Liu, Y., and Yan, H. (2013) A DNA tweezer-actuated enzyme nanoreactor. *Nat. Commun.*, **4**, 2127.

93 Niemeyer, C.M., Koehler, J., and Wuerdemann, C. (2002) DNA-directed assembly of bienzymic complexes from in vivo biotinylated NAD (P) H: FMN oxidoreductase and luciferase. *ChemBioChem*, **3** (2), 242–245.

94 You, M., Wang, R.-W., Zhang, X., Chen, Y., Wang, K., Peng, L., and Tan, W. (2011) Photon-regulated DNA-enzymatic nanostructures by molecular assembly. *ACS Nano*, **5** (12), 10090–10095.

95 Conrado, R.J., Wu, G.C., Boock, J.T., Xu, H., Chen, S.Y., Lebar, T., Turnsek, J., Tomsic, N., Avbelj, M., Gaber, R., Koprivnjak, T., Mori, J., Glavnik, V., Vovk, I., Bencina, M., Hodnik, V., Anderluh, G., Dueber, J.E., Jerala, R., and DeLisa, M.P. (2012) DNA-guided assembly of biosynthetic pathways promotes improved catalytic efficiency. *Nucleic Acids Res.*, **40** (4), 1879–1889.

96 Moon, T.S., Dueber, J.E., Shiue, E., Prather, K.L.J., and Prather, K.L. (2010) Use of modular, synthetic scaffolds for improved production of glucaric acid in engineered *E. coli*. *Metab. Eng.*, **12** (3), 298–305.

97 Delebecque, C.J., Silver, P.A., and Lindner, A.B. (2012) Designing and using RNA scaffolds to assemble proteins in vivo. *Nat. Protoc.*, **7** (10), 1797–1807.

98 Ducat, D.C., Sachdeva, G., and Silver, P.A. (2011) Rewiring hydrogenase-dependent redox circuits in cyanobacteria. *Proc. Natl. Acad. Sci. U.S.A.*, **108** (10), 3941–3946.

99 Schirmer, A., Rude, M.A., Li, X., Popova, E., and del Cardayre, S.B. (2010) Microbial biosynthesis of alkanes. *Science*, **329** (5991), 559–562.

100 Torella, J.P., Ford, T.J., Kim, S.N., Chen, A.M., Way, J.C., and Silver, P.A. (2013) Tailored fatty acid synthesis via dynamic control of fatty acid elongation. *Proc. Natl. Acad. Sci. U.S.A.*, **110** (28), 11290–11295.

101 Barros, L.F. and Martínez, C. (2007) An enquiry into metabolite domains. *Biophys. J.*, **92** (11), 3878–3884.

102 Lee, H., DeLoache, W.C., and Dueber, J.E. (2012) Spatial organization of enzymes for metabolic engineering. *Metab. Eng.*, **14** (3), 242–251.

103 Fu, J., Liu, M., Liu, Y., Woodbury, N.W., and Yan, H. (2012) Interenzyme substrate diffusion for an enzyme cascade organized on spatially addressable DNA nanostructures. *J. Am. Chem. Soc.*, **134** (12), 5516–5519.

104 Ponchon, L., Catala, M., Seijo, B., El Khouri, M., Dardel, F., Nonin-Lecomte, S., and Tisne, C. (2013) Co-expression of RNA-protein complexes in *Escherichia coli* and applications to RNA biology. *Nucleic Acids Res.*, **41** (15), e150.

105 Gibson, D.G., Young, L., Chuang, R.-Y., Venter, J.C., Hutchison, C.A., and Smith, H.O. (2009) Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat. Methods*, **6** (5), 343–345.

106 Kosuri, S., Eroshenko, N., LeProust, E.M., Super, M., Way, J., Li, J.B., and Church, G.M. (2010) Scalable gene synthesis by selective amplification of DNA pools from high-fidelity microchips. *Nat. Biotechnol.*, **28** (12), 1295–1299.

107 Liang, J.C., Bloom, R.J., and Smolke, C.D. (2011) Engineering biological systems with synthetic RNA molecules. *Mol. Cell*, **43** (6), 915–926.

108 Wang, K., Neumann, H., Peak-Chew, S.Y., and Chin, J.W. (2007) Evolved orthogonal ribosomes enhance the efficiency of synthetic genetic code expansion. *Nat. Biotechnol.*, **25** (7), 770–777.

109 Neumann, H., Wang, K., Davis, L., Garcia-Alai, M., and Chin, J.W. (2010) Encoding multiple unnatural amino acids via evolution of a quadruplet-decoding ribosome. *Nature*, **464** (7287), 441–444.

110 Mali, P., Yang, L., Esvelt, K.M., Aach, J., Guell, M., DiCarlo, J.E., Norville, J.E., and Church, G.M. (2013) RNA-guided human genome engineering via Cas9. *Science*, **339** (6121), 823–826.

111 Sachdeva, G., Garg, A., Godding, D., Way, J., and Silver, P.A. (2014) In vivo co-localization of enzyme on RNA-scaffolds increases metabolic production in a geometrically dependent manner. *Nucleic Acids Res.* doi: 10.1093/nar/gku617

112 Douglas, S.M., Bachelet, I., and Church, G.M. (2012) A logic-gated nanorobot for targeted transport of molecular payloads. *Science*, **335** (6070), 831–834.

113 Fu, J. and Yan, H. (2012) Controlled drug release by a nanorobot. *Nat. Biotechnol.*, **30** (5), 407–408.

114 Robinson-Mosher, A., Shinar, T., Silver, P.A., and Way, J. (2013) Dynamics simulations for engineering macromolecular interactions. *Chaos*, **23** (2), 025110.

115 Wu, C.-H., Lockett, M.R., and Smith, L.M. (2012) RNA-mediated gene assembly from DNA arrays. *Angew. Chem. Int. Ed.*, **51** (19), 4628–4632.

116 Lucks, J.B., Mortimer, S.A., Trapnell, C., Luo, S., Aviran, S., Schroth, G.P., Pachter, L., Doudna, J.A., and Arkin, A.P. (2011) Multiplexed RNA structure characterization with selective 2′-hydroxyl acylation analyzed by primer extension sequencing (SHAPE-Seq). *Proc. Natl. Acad. Sci. U.S.A.*, **108** (27), 11063–11068.

117 Shu, X., Lev-Ram, V., Deerinck, T.J., Qi, Y., Ramko, E.B., Davidson, M.W., Jin, Y., Ellisman, M.H., and Tsien, R.Y. (2011) A genetically encoded tag for correlated light and electron microscopy of intact cells, tissues, and organisms. *PLoS Biol.*, **9** (4), e1001041.

118 Martell, J.D., Deerinck, T.J., Sancak, Y., Poulos, T.L., Mootha, V.K., Sosinsky, G.E., Ellisman, M.H., and Ting, A.Y. (2012) Engineered ascorbate peroxidase as a genetically encoded reporter for electron microscopy. *Nat. Biotechnol.*, 1–9.

119 Choi, H.M.T., Beck, V.A., and Pierce, N.A. (2014) Next-generation in situ hybridization chain reaction: higher gain, lower cost, greater durability. *ACS Nano*, **8** (5), 4284–4294.

120 Jungmann, R., Avendaño, M.S., Woehrstein, J.B., Dai, M., Shih, W.M., and Yin, P. (2014) Multiplexed 3D cellular super-resolution imaging with DNA-PAINT and exchange-PAINT. *Nat. Methods*, **11** (3), 313–318.