

# Biomedical applications of RNA-based devices

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## Abstract

Emergent RNA technologies employ sequence and structural information to perform a diversity of biological functions. Synthetic RNA molecules have been developed for a wide array of applications, including genetic regulation, environmental sensing, and diagnostics devices. Recent advances in chemical synthesis and computational design of RNA have enhanced our ability to program novel functions and expand upon current biomedical applications for therapeutics and diagnostics. In this review, we highlight recent advances in synthetic RNA devices that have been engineered for biomedical systems, while addressing the current limitations and challenges of translating these engineered functional RNAs to clinical applications.

## Addresses

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## Introduction

RNA is a versatile biomolecule with significant capacity to encode genetic information, perform catalytic cleavage reactions [1], regulate mRNA stability through RNA interference (RNAi) [2], and adopt three-dimensional structures to bind ligands through hydrogen bonding networks, pi-stacking, and electrostatic interactions [3]. RNA structure is governed by Watson-Crick base pairing rules and thus RNA molecules can be programmed to perform these various functions in biomedical engineering applications, providing differential and orthogonal layers of regulation for virtually any biological target of interest [4]. Given the capacity of RNA molecules to interact with various biomolecular inputs, researchers have coupled environmental sensing with functional

outputs to generate RNA-based devices with input/output control, expanding the availability of synthetic parts for programming biology [5]. Computational tools and *in vitro* library screening methods have enhanced the potential for RNA devices to be rapidly designed, synthesized, assayed, and explored for diagnostics and *in vivo* therapeutic applications, as well as aiding in biomedical discoveries and drug screening [6]. Regulatory RNAs such as microRNAs (miRNAs) and small-interfering RNAs (siRNAs) [2,7], RNA scaffolds and aptamers to bind nucleic acids, small molecules, proteins, and whole cells [8,9], ribozyme-mediated genetic control [10], or recent advances in CRISPR/Cas genome engineering [11] demonstrate the engineering potential of synthetic RNA devices for controlling a diverse array of biomedical targets. As the time and cost of nucleic acid synthesis continues to decrease [12], selection protocols for functional RNA molecules become higher throughput, and therapeutic delivery challenges are addressed, these devices will be more readily deployed in clinical applications.

In this review, we discuss recent advances in RNA device engineering for various biomedical applications, ranging from biomedical diagnostics to potential therapeutic gene circuits. Specifically, we discuss efforts to leverage antisense- and CRISPR-based RNAs for *in vitro* diagnostic devices. We also discuss efforts to use engineered self-cleaving ribozyme and miRNA devices to regulate natural and synthetic gene circuits for applications in cellular and gene therapies. We highlight the design and selection methodologies to generate these devices, the modularity and flexibility inherent in RNA device engineering, and efforts to expand the number of targets and ligands available for controlling biological systems. Finally, we discuss translational efforts to improve the pipeline of RNA devices for clinical applications, the challenges of delivery and methods to improve selection and design of novel functional RNA molecules. Advances in chemical modifications and viral delivery systems will promote biomedical use, while considerations around safety and efficacy are paramount to their ultimate clinical translation.

## RNA devices enable diagnostic platforms with rapid and sensitive detection

While Watson-Crick base pairing rules for RNA nucleotides provide a simple set of defined hydrogen bond interactions (A:U vs C:G), wobble base pairing of G:U, a flexible phosphodiester backbone, and various sugar puckers expand the possible three-dimensional folded structures [3]. SELEX (Systematic Evolution of

Ligands by EXponential Enrichment) relies on the library diversity of a pool of RNA oligonucleotides to recognize any ligand of interest through conformational sampling of the given sequences and identify the tightest binders, generating RNA aptamers that can be used directly as “chemical antibodies” or coupled to other functional RNA molecules to generate environmental sensing devices [13,14]. Given their rapid and cost-effective production and selection, non-immunogenicity, and lower batch-to-batch variation compared to antibodies or other detection methods, RNA aptamers have been explored as novel biomedical diagnostics tools for sensitive and rapid detection of clinically relevant ligands, including small molecule drugs [15], bacterial virulence factors [16], proteins implicated in cancer phenotypes [17–20], and whole cells [21,22]. High-affinity aptamers have been selected for antagonistic activities, and have been employed for diagnostics or conjugated to therapeutic drug vehicles for targeted delivery *in vivo* [14,23].

### **Toehold switches are rapidly designed and deployed nucleic acid diagnostic tools**

RNA technologies have been recently explored as an *in vitro* diagnostics platform for nucleic acids and viral RNA, offering a faster and cheaper detection paradigm for remote and resource-limited areas compared with PCR and antibody-based methods, which are employed clinically in developed nations. Researchers developed a set of RNA regulators called toehold switches that bind *trans*-acting trigger RNA molecules and transduce an encoded biological output *in vivo*, generating novel gene regulatory switches in *Escherichia coli* via rational design [24]. *Cis*-regulatory translational elements, including the Shine-Dalgarno (SD) sequence and start codon, are sequestered in a hairpin structure that is disrupted through cognate RNA binding to the toehold sequence, leading to activation of protein production. Because the binding interaction between the trigger RNA and the toehold switch relies on an antisense linear strand displacement mechanism as opposed to loop-mediated interactions, the kinetic and thermodynamic properties of toehold switches can be predicted [25]. In addition, toehold switches are computationally designed via a forward engineering approach and can be rapidly synthesized, characterized, and deployed as genetic control elements in prokaryotes.

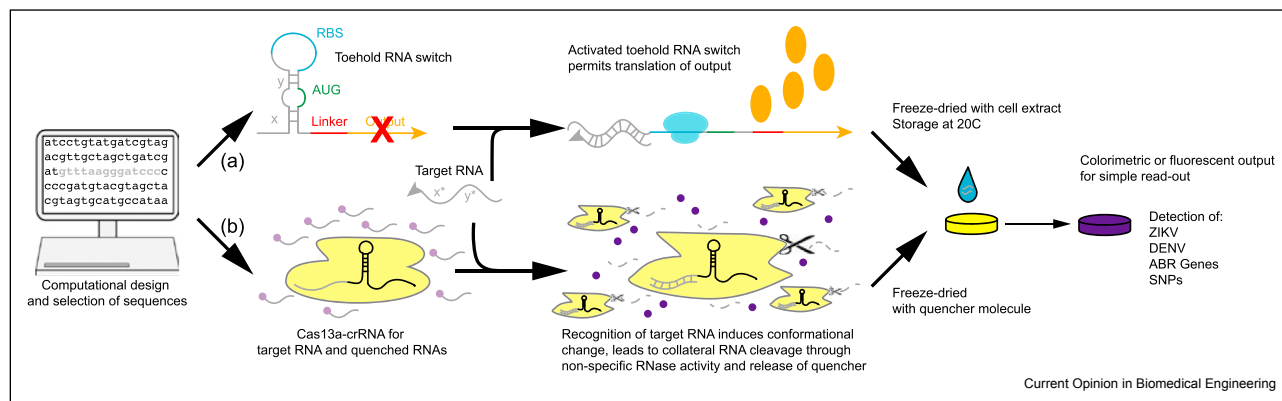
Toehold switches have been recently applied to the design of freeze-dried paper-based synthetic gene networks and diagnostic devices for Zika virus (ZIKV) and Ebola virus (EBOV) [26,27]. Toehold switches are freeze-dried with *E. coli* cell extract, which provides the translational machinery to produce an output indicating the absence or presence of viral RNA. By coupling the presence of ZIKV or EBOV viral RNA with *in silico* designed toehold switches triggering the expression of

LacZ (colorimetric output) or GFP (fluorescence output), diagnostic devices based on simple visual outputs or easy-to-operate optical readers can be deployed (Fig. 1). The switches distinguish between similar strains with single base pair resolution and can be combined with amplification methods to detect low femtomolar amounts of viral RNA in plasma samples. The shelf life and stability of freeze-dried components at room temperature was demonstrated for up to a year, with diagnostic results achievable in 3 h. The paper-based diagnostics based on toehold RNA switches offer several advantages over serological diagnostics in resource-limited areas, while also providing rapid and sensitive detection when PCR-based analysis cannot be employed. The discriminatory power of RNA–RNA sequence interactions with strain-specific viruses, compared to cross-reactive antibodies, is essential for providing proper treatment and mitigating antiviral resistance to RNA viruses [28]. Continued work on increasing clinical sensitivity and expansion to other RNA viruses and parasites will dramatically improve point-of-care health outcomes for infected patients [29,30].

### **RNA-guided CRISPR systems improve sensitivity of nucleic acid detection for diagnostics**

The discovery of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) systems and their associated RNA-guided nucleases has been explored for genome editing given the flexibility in guide RNA (gRNA) design and synthesis [31]. However, given its ability to recognize and cut a specific DNA or RNA sequence, CRISPR has been implicated as a sensitive detection system and diagnostic tool. Rapid molecular diagnostics have been achieved with the type VI CRISPR-Cas13a system to detect small amounts of RNA through its dual RNase activities for pre-crRNA processing and specific recognition of a target RNA sequence and more general non-specific RNase activity for collateral cleavage of proximal non-target nucleic acids [32]. Upon recognition of a specific target sequence by the crRNA bound to Cas13a, the enzyme adopts a catalytically active conformation that cleaves all RNAs, which potentially evolved as a programmed cell death mechanism to prevent viral replication upon infection in prokaryotes [33,34]. Based on this specific and general RNase activity, researchers developed the SHERLOCK (Specific High Sensitivity Enzymatic Reporter UNLOCKing) *in vitro* detection platform for rapid and attomolar-sensitive detection of ZIKV, Dengue Virus (DENV), and bacterial pathogens from clinical isolates with high strain and sequence discriminatory power [35]. Solution and paper-based systems were demonstrated with purified *Leptotrichia wadei* Cas13a (LwCas13a) incubated with gRNA for the target of interest and a quenched fluorescent RNA molecule

Figure 1



**In vitro RNA devices recognize target nucleic acids to produce a diagnostic output in paper-based systems.** Genome sequences and computational design of target sequences produce nucleic acid sensors for viral genomes, SNP variants, or other pathogenic markers. Using toehold switches (a) to produce a protein output or SHERLOCK (b) to collaterally cleave fluorescently quenched RNAs upon recognition of the target viral nucleic acid, freeze-dried paper-based systems allow for simple visual or optical outputs such as colorimetric dyes or fluorescence for sub-nanomolar detection.

Figure adapted from Refs. [26,27,35].

that is collaterally cleaved when Cas13a recognizes its cognate RNA. The sensitivity of the SHERLOCK platform was increased to single-molecule detection through the incorporation of reverse transcription, recombinase polymerase amplification (RPA) and T7 transcription steps to amplify initial amounts of viral RNA. The SHERLOCK platform has also been applied to infer genotype and the presence of low frequency cancer mutations ( $\sim 0.1\%$  allelic fractions) using cell-free DNA samples.

Taken together, the examples highlight the potential of these nucleic acid-based detection systems for developing affordable diagnostics platforms in areas of global health, infectious diseases, and genetic testing. Assays can be developed for novel biomarkers within a few days and paper-based diagnostics can then be rapidly distributed for less than \$1 per test in resource-limited locations or when serological testing is unavailable. However, the isothermal amplification techniques that many of these systems rely on are susceptible to contaminating nucleic acids, producing off-target amplicons and ultimately higher false positive rates that may pose therapeutic challenges in resource-limited regions [36,37]. In addition, the high rates of genetic drift in RNA viruses such as Zika and Ebola, may pose challenges for the design of probes and crRNAs to detect these viruses with the necessary discriminatory power. Toehold switches and SHERLOCK can tolerate some base pair mismatches, which may be useful to still detect the virus of interest, but efficacy may suffer and result in false negatives. Yet, given the ease of computationally assisted design and nucleic acid synthesis, detection platforms based on RNA devices have the

advantage of being rapidly developed and deployed, expanding clinically relevant diagnostic power to developed and developing regions.

### Synthetic RNA devices interface with the environment to conditionally regulate gene expression

Regulating expression of endogenous genes and exogenously delivered transgenes through programmable inputs remains a significant challenge in biomedical applications and clinical translation of biotechnologies [38]. Natural RNA elements provide inspiration for engineering synthetic gene-regulatory devices by leveraging structural cues, catalytic activities, and anti-sense mechanisms of gene silencing with rational design and evolution to select for desired functions [39]. Based on structure and sequence information, RNA devices can be engineered to be responsive to specific molecular inputs, such as small-molecule or protein ligands or other RNA molecules, resulting in gene-regulatory devices that can regulate protein expression without accessory proteins or transcription factors [5,40]. In addition, RNA devices are typically compact (i.e., less than 200 nucleotides long) and can be readily integrated into most genetic constructs.

### Ribozyme switches are a broadly deployed class of ligand-responsive gene-regulatory devices

Ribozyme switches are a class of RNA-based gene-control devices that have been utilized in several engineered cell systems with biomedical relevance. The majority of ribozyme switches described to-date have

been based on coupling the hammerhead ribozyme of the satellite RNA of tobacco ringspot virus (sTRSV) with an RNA aptamer in a manner that achieves ligand control over cleavage activity [5,41], although researchers have also engineered RNA switches based on the Hepatitis Delta Virus [42] and twister ribozymes [43]. In eukaryotic systems, ribozyme switches are typically integrated into the 3' untranslated region (3' UTR) of a gene of interest. The ligand bound state of the switch can either be associated with a ribozyme inactive conformation, in which the ribozyme structure is disrupted, resulting in increased transcript stability and protein production (i.e., a gene expression "ON" switch), or a ribozyme active conformation, resulting in transcript cleavage and subsequent rapid degradation and thus lowered protein production (i.e., a gene expression "OFF" switch) (Fig. 2) [40]. Removing the inducer molecule has been shown to reverse the effects on gene expression state. Greater dynamic range in gene expression (defined as the ratio of gene expression between the ON and OFF states) has been achieved by integrating multiple copies of the ribozyme switch after the gene of interest, although this strategy generally results in a lower basal level of expression [44].

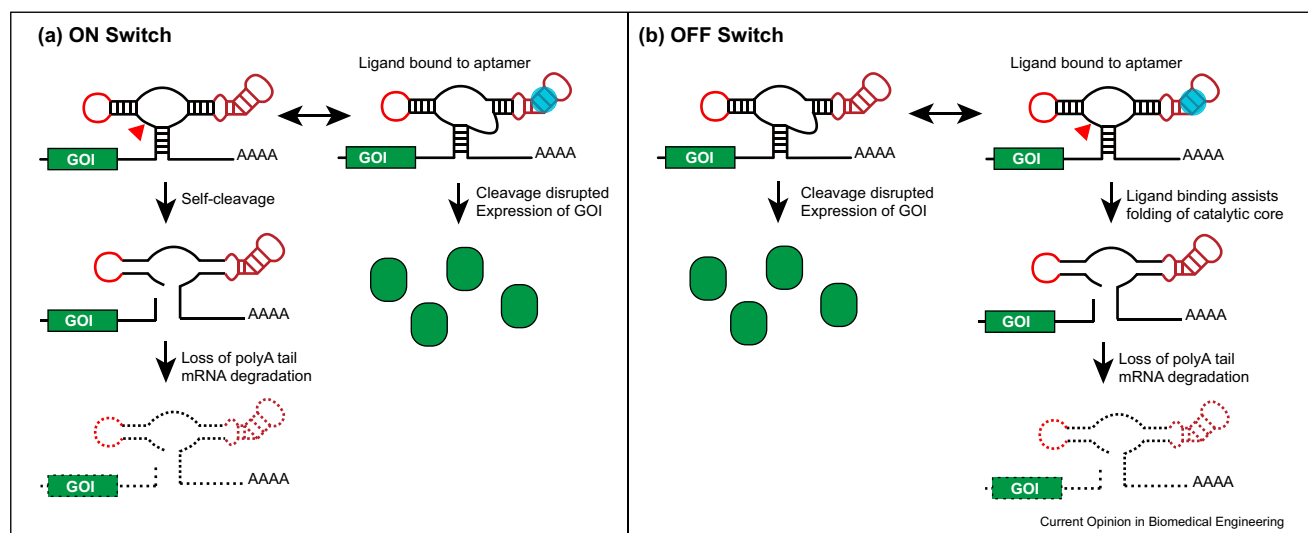
While the modular nature of RNA device architectures support the potential of generating tailored genetic regulators to any ligand of interest, the current set of ligands available for conditional gene expression control

via RNA devices is limited [45]. Most small-molecule ribozyme switches to-date rely on the theophylline or tetracycline aptamers based on their compact structure, well-characterized binding and specificity, and subsequent studies to examine physical coupling strategies with the sTRSV ribozyme [46–49]. Although theophylline and tetracycline are FDA-approved drugs suitable for clinical translation of these ligands, the concentrations required in cell culture models are low millimolar, such that achieving these concentrations through systemic delivery would be highly toxic for any clinical applications of these switches [50]. As a result, these ligand/aptamer pairs have been more relevant for building and validating model systems that can be expanded upon with higher affinity ribozyme switches and further work to improve small-molecule ligands available for genetic control to improve the safety and efficacy of ribozyme switch-based therapeutics is necessary. Protein-responsive ribozyme switches to a diverse set of protein ligands have also been developed for conditional control in mammalian cells, including switches recognizing the MS2 coat protein [51], the E2F1 oncogene [52], beta-catenin [53], and the N-peptide from bacteriophage lambda [54].

### Ribozyme switches control complex cellular growth and division phenotypes

Cellular control with ribozyme switches has been explored for regulating therapeutic circuits of *ex vivo*

Figure 2



**Ribozyme switches recognize diverse ligands to conditionally control target gene expression.** In the ON switch architecture (a), the catalytically-inactive conformation of the ribozyme is associated with ligand binding to the aptamer, preventing self-cleavage and leading to rapid degradation of the mRNA, thereby allowing for increased expression of the gene of interest. In the OFF switch architecture (b), the catalytically-active conformation of the ribozyme is associated with ligand binding to the aptamer, leading to self-cleavage and rapid degradation of the mRNA, thereby allowing for reduced expression of the gene of interest. Small molecule and protein aptamers have been integrated into the ribozyme switch architecture for conditional gene-control in mammalian systems.

Figure adapted from Ref. [60].



genetically modified cells. In an early example, researchers used ribozyme switches to regulate expression of the cytokines IL-2 and IL-15 in T cells to link *in vivo* proliferation to the presence of a drug as a persistence mechanism of transfused cells for adoptive immunotherapies [44]. In this early proof-of-concept demonstration the modularity of the sensor component was demonstrated by replacing the theophylline aptamer with the tetracycline aptamer and the modularity of the device output was demonstrated for two different cytokine transgenes. In a separate example, theophylline-responsive ribozyme switches were used to regulate the expression of herpes simplex virus-thymidine kinase and achieve suicide gene control of tumor cells in the presence of two drug inputs – ganciclovir and theophylline [50].

While these previous examples regulated the highest node of a cellular pathway to induce apoptosis or cell proliferation, ribozyme switches have also been engineered to control complex systems behaviors, such as cell cycle progression through mitosis. Researchers identified key regulatory factors in the cell cycle that arrested cells in either G0/1 phase (p27) or in G2/M phase (cyclinB1 mutant), engineered theophylline-responsive ON ribozyme switches after these transgenes, and integrated these devices into cells for stable expression [55]. Induction with theophylline arrested 77% of the population in G0/1 compared to 59% of uninduced cells, a comparable change to the constitutively expressed p27 control, with a similar result achieved with G2/M arrest - 73% versus 55%. With stable integration of these cell cycle controller devices into the genome, cells maintained cell cycle arrest in the presence of theophylline over six weeks and were generally reversible upon removal of the inducer, although leakiness of the device did affect basal fractions between the cell phases.

### Strategies to increase safety and genetic control of viral vectors with ribozyme switches

Recently, viral vectors have gained more traction for biomedical applications as delivery vehicles for therapeutic genes, treatment strategies for cancer, and vaccines encoding viral antigens to raise immunity against infections [56]. Given its intrinsic *cis*-regulatory mechanism of self-cleavage to regulate gene expression, researchers have explored ribozyme switch-mediated control of viral vectors, as a way to circumvent challenges associated with introducing protein-based genetic regulation in a constricted packaging size [57]. In one example, conditional transgene expression was demonstrated in a replication-deficient adenovirus and replication-competent oncolytic adenovirus when theophylline-responsive ribozyme switches were integrated into the 5'- and 3'-UTRs of the transgenes [58].

The work demonstrates that ribozyme switches can serve as a powerful tool for optimal dosing or as a safety mechanism for viral therapies. In a second example, researchers incorporated theophylline-responsive ribozyme switches into specific locations of a viral genome in order to limit replication efficiency and reduce infectivity of a DNA virus, a negative-strand ssRNA measles virus [57], and in a later study a positive-strand ssRNA alphavirus [59]. By conditionally controlling viral replication with a small molecule, the authors developed a mechanism to achieve safer therapies based on engineered viruses. These safety switches rely on mechanisms different from standard antivirals, and thus would present different evolutionary pressures to resistance. One potential challenge with the approach of using RNA switches to control the infectivity and replication efficiency of RNA viruses is that the higher rates of genetic drift associated with these viruses may favor the selection of mutants that inactivate the switch and regain infectivity, thereby reducing safety and efficacy. However, small fractions of escape mutants that recover infectivity in replication-deficient or limited viruses will likely not reach adverse levels and infection can be eliminated by the immune system before significant risks arise for the patient [59]. Other considerations, such as integrating multiple ribozyme switches for replication control, tropism for specific cell types, and replication-deficient or replication-limited viruses will be essential for clinical translation.

### High-throughput screening generates better ribozyme switches for genetic control

Most RNA switches described to-date are characterized by relatively small dynamic ranges, limiting their use as robust genetic controllers in certain applications. Leveraging high-throughput screening technologies such as FACS and Next-Generation Sequencing (NGS) can be used to generate novel ligand-responsive switches, improve activation ratios, and achieve faster self-cleavage kinetics, all desirable features for improving the gene-regulatory activities of this class of RNA devices. Researchers recently demonstrated a FACS-seq method for generating ribozyme switches in yeast with the above features using an *in vivo* FACS-seq platform and a tertiary loop–loop interaction architecture as opposed to earlier architectures based on secondary structure interactions [60]. The method integrates libraries of ribozyme switches into the 3' UTR of a GFP reporter in a two-color plasmid system, where a mCherry reporter is used as a control to account for cell-to-cell variability in gene expression. Cells transformed with the library plasmids are grown in the presence and absence of the ligand and each cell population is sorted into discrete bins based on the ratio of GFP to mCherry expression. The library members in each bin are recovered, amplified with

unique barcodes, and NGS is used to count the number of times a unique sequence occurs in each bin. From these counts, the gene-regulatory activity is reconstructed for each library member. The FACS-Seq method thus provides activity data for all members of the library, allowing for the identification of highly functional switches as well as improved insight into the overall design space for this important class of RNA devices.

Given that ribozyme switches tested and analyzed in yeast have correlative activity in mammalian cells [61], yeast can serve as a rapid prototyping model organism for mammalian RNA switches. However, selection and optimization of ribozymes switches in mammalian cells may be better suited for biomedical translation to capture the transcriptional dynamics and intracellular environment of the appropriate host cell. Researchers have also performed ribozyme switch selections in a luciferase plate-based assay in mammalian cells [62], which exhibits a much lower throughput than a FACS-based screening approach. A combination of high throughput or automated screening and selection of ribozyme switches in mammalian cells for novel ligands with less toxicity, improved dynamic range, and faster kinetics will contribute to the translation of this important class of gene-regulatory devices to biomedical applications.

### miRNA-responsive switches rely on cell-specific cues to activate a synthetic RNA device

RNAi is an endogenous regulatory system that has been used to advance biomedical discoveries, target-drug screens, gain/loss-of-function studies, and new therapeutics [2,4]. Natural miRNA and siRNA silencing activity is based on sequence complementarity to a target transcript – miRNAs with partial complementarity lead to translational repression, whereas siRNAs that exhibit full complementarity direct endonucleolytic cleavage of the target transcript via RISC and AGO2. Endogenous miRNA profiles have been used to classify [63] and direct expression of target genes to specific cell populations [64]. Cell type-specific miRNA profiles and miRNA dysregulation in cancer cells provide unique signatures that when combined with endogenous RNAi machinery can be processed by RNA devices to repress or silence genes of interest in targeted cell populations. In an early example, researchers demonstrated that characteristic miRNA profiles in HeLa cells can be detected in a multi-logic gate circuit (or miRNA classifier device) such that apoptosis is induced when the anticipated miRNA profile is detected (Fig. 3) [63]. Leveraging cell-specific miRNA profiles to activate a therapeutic circuit will reduce off-target effects and increase safety of these therapies for systemic delivery.

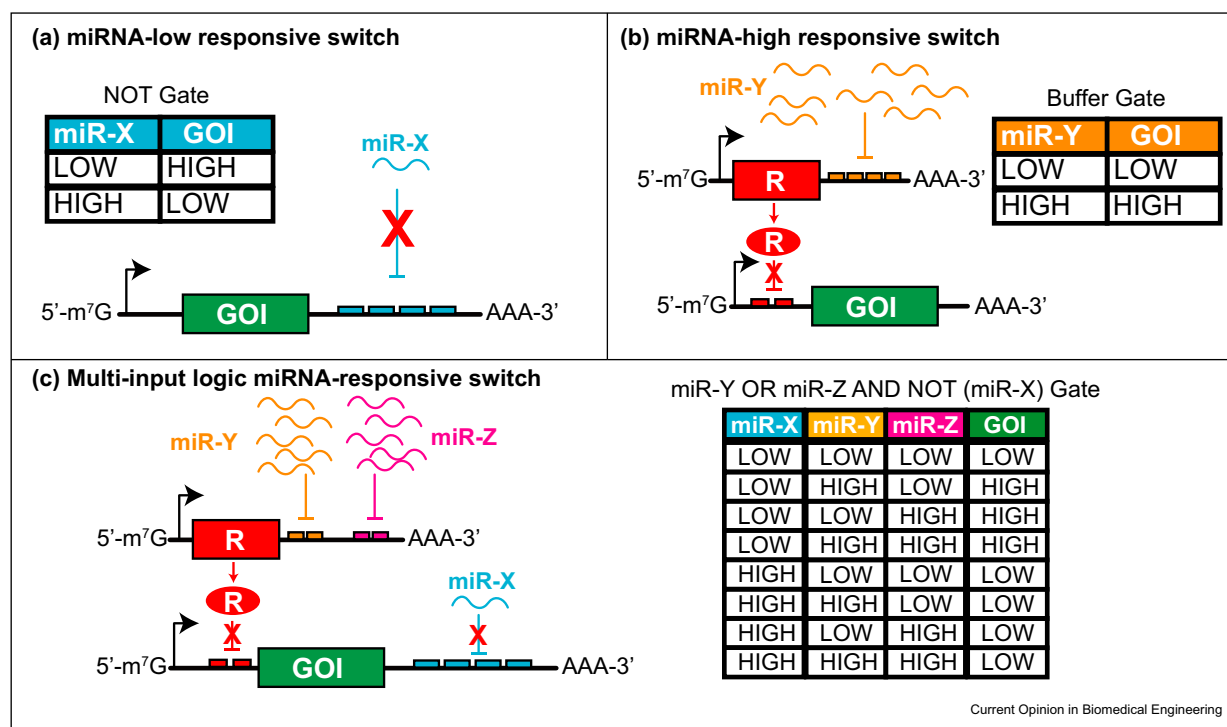
One recently explored application of such miRNA classifier devices has been for exogenous RNA-only circuits and mRNA therapies for non-viral delivery of post-transcriptional gene products [65]. mRNA therapies provide transient control of gene expression without random genome integration typical of conventional gene therapies. Researchers explored an RNA-only classifier circuit that induced apoptosis preferentially in HeLa cells by recognizing high levels of miR-21 and low levels of miR-141, miR-142(3p), and miR-146a in a two-part repression cascade. Production of modified RNA (modRNA) devices with a polyA tail and 7-methylguanylate cap allowed for stability and direct translation of mRNA to proteins, with miRNA targeting sites incorporated in the 3' UTR to direct expression to the desired cell type. In a follow-up study using a similar architecture, a miRNA-responsive switch system was demonstrated to induce Cas9 gene editing in HeLa and human induced pluripotent stem cells (hiPSCs) using cell marker-specific miRNAs [66]. This additional consideration for genome editing can eliminate off-target DNA cleavage in non-target cell populations, a concern for safety of *in vivo* therapeutics.

A similar approach was used to purify a heterogeneous hiPSC population into differentiated cell types by transfecting a modRNA encoding an apoptotic gene and miRNA targeting sites in the 5' UTR corresponding to highly abundant miRNAs for that specific cell type. The researchers identified miRNAs present in hiPSC-derived cardiomyocytes, hepatocytes, endothelial cells, and insulin-producing cells to include sites targeting these miRNAs in the modRNA design in order to purify differentiated cells with >95% efficiency [67]. Cardiomyocytes selected with miR-1 and miR-208a-responsive switches exhibited zero incidences of tumorigenesis when transplanted into mouse hearts, highlighting efficient purification and translation for other cell types with difficult isolation protocols. Delivering an autonomous apoptotic circuit to purify differentiated cells from a heterogeneous population reduces the need for cell sorting techniques that often kill many cells, when large numbers of viable cells are essential for regenerative cell therapies.

### Conclusions and future directions

One of the key advantages of RNA-based devices are their ability to recognize a diverse range of ligands, spanning nucleic acids, small molecules, and proteins, to transduce a desired biological output. Rational engineering aided by computer design supports rapid prototyping and validation to generate RNA molecules responsive to new inputs, particularly other nucleic acids where Watson-Crick base pairing dictates the desired interaction. However, engineering novel ligand-binding aptamers to clinically relevant small molecule and protein inputs, that can be used in designing RNA

Figure 3



**miRNA-responsive switches respond to high- or low-levels of endogenously expressed miRNAs.** In the miRNA-low switch (NOT gate) architecture (a), low levels of miRNA do not significantly inhibit mRNA translation, allowing for production of the protein of interest. In the miRNA-high switch (Buffer gate) architecture (b), a double-inversion module is employed, where high levels of endogenous miRNAs inhibit the production of a repressor protein, allowing mRNA translation of the gene of interest. miRNA-low and high switch designs can be multiplexed into multi-input higher-order logic gates (c), which encode protein production under specific patterns of miRNA expression levels characteristic of a specific cell type of interest. Figure adapted from Refs. [63,65].

switches with desirable properties for biomedical translation, such as low-toxicity, high-affinity, and rapid kinetics, has proven to be more challenging. Advances in high-throughput screening will aid future development efforts in order to expand the number of clinically relevant ligands that RNA devices can respond to. RNA switches typically exhibit a narrower dynamic range of gene expression when compared to highly engineered transcription factor-based systems. However, recent work has demonstrated that the application of computational folding tools [68], *in vivo* screening methods [62], and incorporation of genetic amplification strategies can improve the gene-regulatory performance of RNA devices [69].

The use of RNA-only devices as *in vivo* biosensors or therapeutics can face challenges resulting from short half-lives and potential immune stimulation [70]. While the use of modRNAs can overcome some of these limitations for mRNA transfections, they may run into challenges with long-term stability and robust expression in human therapeutics. RNA chemical modifications, including those of the 2' hydroxyl group or the phosphodiester backbone, have been used to prevent

recognition by endogenous RNases and immune stimulation and have been extensively explored for therapeutic RNAi agents [70,71] and more recently for Cas9 gRNA engineering [72,73]. Such chemical modification approaches will be important for mRNA-based therapeutics to improve the delivery and transient expression of target genes.

RNA devices possess natural functions and engineered modalities that can be harnessed *in vitro* and *in vivo* for a variety of biomedical applications. The modularity, diversity of function, and relative ease of synthesis and design of RNA devices make these functional biomolecules important tools for biomedical engineering and clinical applications.

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## Conflicts of interest

None declared.

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- \* of special interest
- \*\* of outstanding interest

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