Chapter 1

Introduction
Cancer research in the past decades has advanced our mechanistic understanding of the heterogeneity in disease presentation and prognosis observed in patients with similar cancers. These insights have highlighted the importance of tailoring treatment strategies to specific cancer subtypes to increase clinical efficacy. However, the identification of effective biomarkers and the development of personalized cancer treatment using protein- or small-molecule-based pharmaceuticals have proved challenging. Furthermore, non-targeted treatment options such as chemotherapy and radiation, which pose significant systemic toxicities due to a lack of target specificity, remain the standard care for cancer patients. Immunotherapy, which seeks to redirect the exquisite specificity of the immune system against otherwise intractable diseases, has been proposed as an alternative approach to addressing the complex challenge of cancer treatment.

Immunotherapy is a broad treatment paradigm that elicits prophylactic or therapeutic responses by modulating the immune system. In particular, cancer immunotherapy focuses on stimulating the immune system to recognize and destroy tumor growth. Several immunotherapeutic strategies, most notably monoclonal antibody-based agents, have achieved adequate safety and efficacy for clinical applications. Provenge™, a dendritic cell-based vaccine against prostate cancer, recently became the first United States Food and Drug Administration (FDA)--approved therapeutic cancer vaccine, providing powerful support for cell-based immunotherapy as a feasible cancer treatment option. Despite these recent advances, the number of conditions curable by immunotherapy remains limited, and alternative strategies such as
the adoptive transfer of tumor-targeting T cells are under active investigation as potentially more versatile treatment options\textsuperscript{10-12}.

**Adoptive T-Cell Therapy: Potentials and Current Limitations**

T cells are lymphocytes integral to the adaptive immune response in humans and other higher-level organisms. Several types of T cells—including various helper T cells (T\textsubscript{H} cells), regulatory T cells, and cytolytic T cells (CTLs)—patrol the body for the presence of foreign antigens that match specific T-cell receptors (TCRs) expressed on T-cell membranes. T\textsubscript{H} cells and CTLs are activated upon antigen recognition and, together with other components of the immune system, initiate signaling cascades to mount a vigorous defense against foreign infection\textsuperscript{13}. One of the central events in a coordinated immune response is the clonal expansion of CTLs that recognize the particular foreign antigen present in the body. CTLs form conjugates with their targets via receptor recognition, and target-bound CTLs undergo cytoplasmic rearrangements to align intracellular, electron-dense storage granules with the target cell interface. Pore-forming proteins called perforins and serine proteases called granzymes are then released into the CTL-target cell junction, thus effecting target-specific cell destruction\textsuperscript{13,14} (Figure 1.1).
TCRs dictate the specificity of target recognition and destruction by CTLs. Tumor-infiltrating lymphocytes (TILs) found in select cancer patients are naturally occurring CTLs with tumor-targeting TCRs. Autologous TILs can be isolated from patients, expanded \textit{ex vivo}, and reintroduced into the same patients by adoptive transfer to boost the immune response against cancerous growths\textsuperscript{15, 16} (Figure 1.2). However, the isolation and expansion of TILs are time and resource intensive, and not all cancer patients have autologous TILs. As an alternative, the engineering of artificial TCRs provides a mechanism for redirecting CTL activity against otherwise unrecognized targets such as tumor cells\textsuperscript{17}. For example, the Jensen Laboratory has engineered T cells to express chimeric receptors that specifically target tumorigenic growth in glioblastoma multiforme\textsuperscript{18} and neuroblastoma\textsuperscript{19}. In this strategy, CTLs harvested from the patient are genetically modified to express the chimeric receptor, expanded \textit{ex vivo}, and re-administered to the patient to elicit tumor-specific cytolytic activity\textsuperscript{20} (Figure 1.2). Pilot studies have demonstrated the safety of engineered T cells as a treatment option\textsuperscript{21, 22}.
However, sustaining T-cell survival after adoptive transfer remains a major challenge for both natural TILs and engineered T cells, and multiple clinical trials have shown that the efficacy of adoptive T-cell therapy in humans is often limited by the failure of transferred T cells to survive in the host\textsuperscript{23-25}.

**Adoptive Transfer of Autologous Tumor-Infiltrating Lymphocytes (TILs)**

- **Figure 1.2.** Schematic of adoptive T-cell transfer using either autologous TILs or engineered tumor-targeting T cells. (A) Autologous TILs are cultured from resected tumor specimens with exogenous interleukin-2 (IL-2). (B) Isolated TILs are expanded *ex vivo*. (C) Expanded TIL populations are reintroduced into the patient. (D) A heterogeneous population of T cells is harvested from peripheral blood samples of the cancer patient. (E) Isolated T cells are genetically modified to express tumor-targeting receptors. (F) Engineered tumor-targeting T cells are expanded *ex vivo*. (G) Expanded engineered T-cell populations are reintroduced into the patient.

The survival and proliferation of T cells following adoptive transfer is constrained by the limited availability of homeostatic cytokines (interleukin (IL)-15/IL-7) and stimulatory antigen presenting cells. Pre-transfer lymphodepletion, which removes pre-existing cytokine-consuming lymphocytes, combined with post-transfer administration of high-dose IL-2 has been shown to significantly improve the persistence of adoptively
transferred tumor-infiltrating lymphocytes\textsuperscript{26}. However, such treatments require that the patients be subjected to total body irradiation/chemotherapy and toxic levels of IL-2. Alternative strategies based on the unregulated expression of growth-related genes have been developed to prolong T-cell survival, including expression of the anti-apoptotic genes \textit{bcl}-2 and \textit{bcl}-\textit{x}\textsubscript{L}, overexpression of the human telomerase reverse transcriptase (\textit{hTERT}) gene, and expression of genes encoding the growth factors IL-2 and IL-15\textsuperscript{27}. While capable of sustaining T-cell survival, these strategies also pose the risk of uncontrolled lymphoproliferation and leukemic transformation. Therefore, the ability to integrate growth-stimulatory gene expression with tightly controlled genetic regulatory systems could greatly improve the safety and efficacy of adoptive T-cell therapy. The emerging field of synthetic biology provides useful conceptual and technical tools for the construction of such regulatory systems.

**Synthetic Biology: Constructing Novel Devices with Biological Parts**

The advent of enabling technologies such as rapid and affordable DNA synthesis and sequencing, high-throughput protein screening and characterization, and computational approaches to large-scale bioinformatics has transformed biological research and made possible efforts to not only understand nature, but also adopt, improve, and reprogram natural systems to perform human-defined functions. Accordingly, researchers have begun developing new tools for the manipulation and control of cellular components and their functional outputs, leading to the emergence of synthetic biology as a new discipline in biological research and engineering\textsuperscript{28}. 
The concept that novel biological systems can be constructed by recombining existing biological parts—much as new plant hybrids can be generated by grafting parts of existing species—is not unfamiliar to science. However, the ability to efficiently identify, produce, and recombine biological parts at the genomic level was made possible by relatively recent developments of the aforementioned enabling technologies. The tools of synthetic biology have the potential to construct novel genetic devices with functions that are unknown to or difficult to co-opt from nature. For example, synthetic devices capable of inducing gene expression oscillation\textsuperscript{29}, performing light-sensitive edge detection\textsuperscript{30}, and directing bacterial migration in response to the presence of herbicides\textsuperscript{31} have been developed in recent years. Synthetic biology techniques have also been applied to the construction of diverse control devices capable of gene expression regulation, thus providing new toolsets for the engineering of biological systems with fine-tuned functions.

**RNA as the Design Substrate for Synthetic Control Devices**

Biological control devices such as inducible promoter systems have been studied intensively in the past decades, and various protein-based systems capable of information processing have been developed\textsuperscript{32-34}. However, the therapeutic use of protein-based regulatory strategies has been constrained by immunogenicity of heterologous protein components, toxicity of the narrow selection of compatible input molecules, and limited ability to tune the regulatory response. Furthermore, the need to stably express multiple heterologous protein components poses a significant challenge in medical applications. Inspired by the diverse functional roles exhibited by regulatory RNAs in natural
systems and the relative ease by which RNA can be modeled and designed, researchers have begun developing synthetic RNA-based regulatory devices as alternative genetic control strategies.

RNA is composed of four ribonucleotide bases whose interactions through hydrogen bonding, base stacking, and electrostatic interactions are well understood and aptly modeled by freely available softwares. Furthermore, the ability of compact, non-protein-coding RNAs to perform gene expression regulation through a variety of mechanisms—including transcription termination, translational inhibition, and post-transcriptional modifications such as mRNA cleavage and alternative splicing—renders RNA a versatile substrate for the design and construction of synthetic control devices. The small footprint and non-coding nature of regulatory RNAs avoid the problems of multiple-component integration and immunogenicity associated with protein-based systems, making RNA-based devices particularly well suited for therapeutic applications.

Frameworks for Constructing RNA-Based Control Devices

Although a variety of RNA devices capable of programming biological functions have been developed, most devices can be deconstructed into three functional components: sensors, actuators, and transmitters. The sensor component detects an input signal, which could be a molecular ligand or a change in environmental cues such as temperature, and translates the signal into a downstream regulatory function executed by the actuator component, such as post-transcriptional modification or translation inhibition. The sensor component in most RNA devices designed to date consists of aptamers, which are nucleic acid sequences capable of high-affinity binding to specific
ligands, including small molecules, nucleic acid sequences, proteins, and whole cells\textsuperscript{44}. Aptamers to specific ligands of interest can be generated \textit{de novo} using various \textit{in vitro} selection methods\textsuperscript{45-48}, thus allowing for a large variety of ligands to serve as potential input signals. The actuator component varies widely among RNA devices and consists of one or several functional elements, including but not limited to catalytic RNAs such as self-cleaving hammerhead ribozymes (HHRzs), RNA interference (RNAi) substrates such as microRNAs (miRNAs) and short hairpin RNAs (shRNAs), and RNA sequences containing a ribosome binding site (RBS), which could be obscured or exposed in response to signal detection by the sensor component\textsuperscript{41}.

RNA devices can be constructed by direct coupling between the sensor and actuator components. In one example, a theophylline-responsive RNA switch is generated by connecting the theophylline RNA aptamer to an shRNA actuator\textsuperscript{49}. Theophylline binding to the aptamer inhibits Dicer processing of the shRNA and prevents gene expression knockdown mediated through the RNAi pathway. The device thus serves as an “ON switch,” which responds to the presence of molecular input by increasing, or turning on, gene expression. An alternative construction strategy requires a transmitter sequence inserted between the sensor and actuator components for switch function. In one example, the theophylline RNA aptamer is coupled to an RBS sequence preceding the \textit{cheZ} gene via a randomized linker sequence\textsuperscript{50}. Utilizing \textit{cheZ}’s role in regulating bacterial cell tumbling and chemotaxis, researchers were able to screen for linker sequences that confer ligand-responsive control over ribosome access to the RBS by measuring cell motility in a plate-based assay, ultimately isolating a theophylline-responsive RNA device capable of regulating phenotypic output in bacteria.
Although several functional RNA devices have been created by the construction methods described above, both architectures pose sequence and structural restrictions that require extensive modifications for each new device and limit the modularity of the device framework. For instance, regulatory activity of the theophylline-responsive shRNA switch is highly sensitive to the distance between the sites of ligand binding and Dicer cleavage, such that single-base-pair changes can abolish device function\(^{49}\). In the second device, switch activity depends on sequence base-pairing between the sensor and actuator components despite the presence of a transmitter domain. As a result, both architectures would require complete redesigns for the incorporation of new sensor components, and neither can be easily tuned for regulatory stringency due to the limited number of sequence combinations that can produce functional devices.

As an alternative, RNA devices can include an independent transmitter component that translates signal detection by the sensor to functional output by the actuator. In this architecture, the transmitter component provides structural and functional insulation between the sensor and actuator, thereby allowing greater flexibility in the pairing of sensor and actuator components. An example of this construction method is the ribozyme switch platform previously developed in the Smolke Laboratory\(^{51, 52}\).

**Ligand-Responsive Ribozyme-Based Regulatory Devices**

Win and Smolke have reported a platform for the construction of ribozyme switches whose components are modularly coupled and thus adaptable to application-specific requirements\(^{51}\). These devices are composed of three functional domains: a sensor component consisting of an RNA aptamer, an actuator component consisting of a
satellite RNA of tobacco ringspot virus (sTRSV) hammerhead ribozyme, and a transmitter component consisting of a rationally designed RNA sequence that links the sensor and actuator components (Figure 1.3A). The sensor component can be systematically altered to accommodate the desired aptamer (and thus the molecular input specificity) without modifications to the remaining device components. The sTRSV hammerhead ribozyme is an RNA sequence capable of self-cleavage under specific conformations. In the ribozyme-based device, the actuator samples at least two different conformations, only one of which allows ribozyme cleavage. Ligand binding by the sensor component changes the relative thermodynamic stability of the different conformations and alters the rate of change from one conformation to the next, thus affecting the ribozyme’s cleavage activity. The transmitter component relays ligand-binding information from the sensor to the actuator, encodes competitive hybridization events to specify the signal processing function (ON or OFF switch), and contributes to performance tuning of the device. The ribozyme switch is integrated into the 3’ untranslated region (UTR) of the target gene, where ribozyme cleavage results in rapid degradation of the target transcript and downregulation of gene expression. In an ON switch, ligand binding prevents ribozyme cleavage, thereby preserving the target transcript and upregulating gene expression (Figure 1.3B). Conversely, in an OFF switch ligand binding results in the downregulation of gene expression. In addition to single-input switch devices, ribozyme-based devices capable of higher-order computations have been developed, including AND, OR, NOR, and NAND gate devices with functionalities demonstrated in the eukaryotic model organism *Saccharomyces cerevisiae*. 
Figure 1.3. Schematic for a ligand-responsive ribozyme-based ON switch. (A) Ribozyme switches are composed of an RNA aptamer (sensor) and a self-cleaving hammerhead ribozyme (actuator) modularly coupled through a rationally designed linker sequence (transmitter). (B) Switches are designed to sample alternative structural conformations. The most thermodynamically stable conformation changes with the availability of the cognate ligand molecule. An ON switch prefers the ribozyme-active conformation in the absence of ligand molecules, a state in which ribozyme cleavage removes the poly-A tail and subjects the transcript to rapid degradation, resulting in gene expression knockdown (OFF state). In contrast, ligand binding to the sensor component stabilizes the ribozyme-inactive conformation, thus preserving the transcript and upregulating gene expression (ON state).

The modularity and tunability of these ribozyme switches as ligand-responsive gene-regulatory devices have been demonstrated in yeast culture\textsuperscript{51}. For example, switch functionality is preserved and the input responsiveness appropriately altered when the sensor domain is changed from the theophylline aptamer to the tetracycline aptamer. Furthermore, switches exhibiting various knockdown efficiencies and dynamics have been constructed based on rational design strategies that modify the switches’ thermodynamic properties. Specifically, by tuning the sequence of the transmitter component, one can control the absolute stability of each secondary structure and the energetic differences between the ribozyme-active and -inactive conformations of the switch. For example, a ribozyme switch can be designed to have greater knockdown activities by stabilizing the ribozyme-active (i.e., cleavable) conformation relative to the ribozyme-inactive (i.e., non-cleavable) conformation.
In addition to ribozyme-based regulatory designs, the RNAi pathway also holds promise as an RNA-based mechanism for gene expression regulation in diverse organisms. Modularly composed, ligand-responsive switch devices utilizing miRNAs and shRNAs as actuator components have also been developed in the Smolke Laboratory.55, 56

Ligand-Responsive RNAi-Based Regulatory Devices

RNAi is a gene-silencing pathway first recognized in Caenorhabditis elegans as a biological response to exogenous double-stranded RNA (dsRNA). In this pathway, RNAi substrates including miRNAs and shRNAs are processed by endogenous RNases into 21-23-nt double-stranded RNA sequences. Alternatively, double-stranded short interfering RNAs (siRNAs) can be synthesized and used directly without the need for RNase processing. One of the two RNA strands is selectively incorporated into the RNA-induced silencing complex (RISC), thereby activating the protein complex. Activated RISC recognizes its regulatory target by sequence complementation to the loaded miRNA guide strand, and it silences target gene expression by either direct cleavage or translational inhibition of the target transcript.58

Due to its ease of use, RNAi has quickly become a standard tool for sequence-specific, post-transcriptional gene silencing in biological systems ranging from plants to mammalian cells.59, 60 Rationally designed miRNA sequences that resemble naturally occurring miRNAs in structure but carry altered stem sequences targeting specific genes of interest have been shown to inhibit gene expression in human cells.61 Synthetic shRNAs have also been engineered to achieve targeted gene silencing in mammalian
systems\textsuperscript{62, 63}. Since RNAi substrates cause cleavage in \textit{trans}, it is possible to silence both endogenous and transgenic expression through the RNAi pathway\textsuperscript{63, 64}.

The Smolke Laboratory has developed modular and tunable platforms for the construction of small-molecule-responsive shRNA\textsuperscript{55} and miRNA\textsuperscript{56} switches. A strand displacement strategy is used to construct shRNA switches, which operate through the interaction of three components: an RNA aptamer (sensor) is coupled to the terminal loop of an shRNA (actuator) by a competing strand (transmitter) (Figure 1.4). The shRNA switch has at least two accessible conformations, in which the shRNA stem is either properly base-paired or disrupted by the competing strand. Only the properly formed shRNA can be processed by the RNase Dicer and proceed through the RNAi pathway. Similar to the ribozyme switches discussed previously, shRNA switches sample alternative conformations based on the relative thermodynamic stability of each accessible conformation. Ligand binding stabilizes one conformation over the others, thus changing the equilibrium distribution of conformations and allowing ligand-responsive gene expression modulation. Characterization studies performed in human embryonic kidney (HEK) 293 cells have demonstrated the modularity and tunability of shRNA switches, which can be rationally designed to respond to various molecular ligand inputs and generate gradient outputs in response to varying input concentrations\textsuperscript{55}.
Figure 1.4. Schematic for a ligand-responsive shRNA-based ON switch. (A) shRNA switches are composed of an RNA aptamer (sensor) coupled to an shRNA (actuator) through a competing strand (transmitter) that translates ligand binding in the aptamer to conformational changes in the shRNA. (B) Switches are designed to sample alternative structural conformations. The most thermodynamically stable conformation changes with the availability of the cognate ligand molecule. An ON switch prefers the shRNA-active conformation in the absence of ligand molecules, leading to shRNA processing by the RNase Dicer and RNAi-mediated knockdown of the target gene (OFF state). In contrast, ligand binding to the sensor component stabilizes the shRNA-inactive conformation, thus abrogating the RNAi pathway and upregulating gene expression (ON state). This figure is adopted from Ref. 55.

In addition to shRNA switches, ligand-responsive miRNA switches have been developed in HEK 293 cells\textsuperscript{56} (Figure 1.5). In this system, an RNA aptamer is integrated in the basal segment of a miRNA. Ligand binding to the aptamer imposes a constrained structure in the basal segment, thereby preventing miRNA processing by the RNase Drosha and abrogating gene-silencing activity by the RNAi pathway. Similar to the ribozyme and shRNA switch platforms, miRNA switches are modularly composed and can be systematically modified to respond to various molecular inputs. Furthermore, combinatorial expression strategies can be employed to generate multiple-copy miRNA constructs that mimic natural miRNA clusters and improve regulatory stringency. Importantly, miRNA switches can regulate gene expression both in \textit{cis} and in \textit{trans}. By specifying the miRNA sequence to match the target gene, miRNA switches can direct
gene expression knockdown in *trans* through the RNAi pathway. In addition, the miRNA switch can be inserted in the 3’ UTR of a target gene. Processing by the RNase Drosha results in excision of the miRNA from the mRNA, leading to rapid transcript degradation and *cis*-acting gene expression knockdown. Therefore, miRNA devices can be designed to either silence one target gene through two regulatory mechanisms to increase regulatory stringency or simultaneously target two different genes in response to one molecular input.

![Figure 1.5](image.png)

**Figure 1.5.** Schematic for a ligand-responsive miRNA-based ON switch. (A) miRNA switches are composed of an RNA aptamer (sensor) integrated in the basal segment of a miRNA (actuator). (B) In the absence of ligand, the basal segment has a flexible structure that allows recognition and cleavage by the RNase Drosha, thus enabling proper miRNA processing and RNAi-mediated knockdown of the target gene (OFF state). Increasing ligand concentration increases the likelihood of ligand binding to the sensor component, which results in a constrained structure in the basal segment of the miRNA. This structure inhibits Drosha processing, prevents RNAi-mediated gene silencing, and upregulates gene expression (ON state). The figure is taken from Ref. 56.

The ribozyme, shRNA, and miRNA switches described above represent modular and versatile devices with which integrated regulatory systems may be constructed. Although the three platforms act through different mechanisms, they share the same requirement for ligand-sensing activity. Namely, RNA aptamers specific to the molecular input of interest must be available for the construction of switches responsive to the
desired ligand. Therefore, the ability to generate RNA aptamers to the appropriate molecular inputs is critical to the development of RNA-based control devices for diverse applications.

**Aptamers and In Vitro Selection Schemes for Novel Ligand-Binding Sequences**

Aptamers are nucleic acid species that bind to ligands with high affinity and specificity. While nucleic acid species with ligand-binding activities exist in nature, the expanding repertoire of aptamers is largely a product of in vitro selection schemes that have become possible with the development of chemical DNA synthesis, the isolation of reverse transcriptase, and the invention of polymerase chain reaction (PCR). SELEX (Systematic Evolution of Ligands by EXponential enrichment) is the most widely used method for the in vitro selection of DNA and RNA aptamers. In this procedure, a pool of $10^{13}$–$10^{15}$ unique DNA sequences is amplified by PCR from chemically synthesized DNA templates with a randomized region flanked by fixed primer sequences. For RNA aptamer selection, the DNA library is used as a template for in vitro transcription to generate an RNA sequence pool. This RNA library is incubated with the ligand of interest, and the ligand-bound pool is isolated by separation techniques such as column chromatography and membrane filtration. The bound species are collected, reverse transcribed, amplified by PCR, and subjected to iterative rounds of selection following the same protocol. Increased selection pressure may be applied with each cycle to alter the properties of the resultant sequence pool. Such strategies include lowering ligand concentrations or increasing wash volumes to increase binding affinities, lowering Mg$^{2+}$ content in the selection buffer to decrease ion concentration dependence, and
performing appropriate negative selections against related ligand species to tune the specificity of the resultant aptamer pool.

Aptamer sequences to diverse molecular targets have been generated since the advent of SELEX and other in vitro selection methods in the early 1990s. However, the aptamers reported thus far have mainly been selected for in vitro sensing applications, and many of the existing aptamers are not optimized for activity under physiological conditions. As a result, the great majority of ligand-responsive RNA devices developed to date have relied on the use of a few well-behaved aptamers—particularly those specific to the small molecules theophylline and tetracycline, both of which have considerable cytotoxicity—thus limiting the range of potential applications for these devices. The generation of novel aptamers to clinically suitable molecular targets will enable the construction of RNA-based control devices with diverse applications in health and medicine. In particular, the ability to regulate cellular functions with RNA-based devices can address critical areas for improvement in cell-based immunotherapy.

**Synthetic RNA-Based Regulatory Systems for T-Cell Proliferation Control**

As discussed above, adoptive T-cell therapy is a promising paradigm for cancer treatment, but its safety and efficacy is dependent on the ability to precisely orchestrate cellular behaviors—particularly proliferation—in vivo. The challenge of reprogramming the behaviors of complex biological processes is an area of active research in the field of synthetic biology, and of RNA engineering in particular. The development of RNA-based control devices to date has largely focused on the exploration of individual regulatory mechanisms and the construction of stand-alone devices. This body of knowledge now
enables the development of integrated control systems that can interface with genetic networks and signaling pathways to achieve regulatory functions at a systems level.

With the aim of improving the safety and efficacy of adoptive T-cell immunotherapy, we set out to develop regulatory systems capable of T-cell proliferation control through the use of small molecule–responsive, RNA-based regulatory devices. The paramount criterion of patient safety demands stringent control over the growth of transferred T cells in vivo. Conversely, therapeutic efficacy requires robust T-cell proliferation during the treatment period. To achieve both objectives, we focused our efforts on modulating the IL-2 and IL-15 cytokine signaling pathways central to T-cell proliferation, and developed multiple control systems to provide regulatory redundancies that ensure effective control over the fate of transferred T cells in vivo. Utilizing both ribozyme- and miRNA-based control devices, we developed synthetic regulatory systems capable of genetic control over both transgenic, growth-promoting cytokines and endogenous cytokine receptor chains. To further expand the applicability of our RNA-based regulatory systems in clinical settings, we also explored the selection of novel RNA aptamers specific to clinically suitable small molecules to serve as the sensor components of ribozyme- and miRNA-based control devices.

**Regulatory System Development and Thesis Organization**

In this thesis, we present the development of RNA-based regulatory systems capable of ligand-responsive control over T-cell proliferation. In Chapter 2, we discuss the translation of ribozyme switches from yeast to mammalian hosts and examine design parameters that are critical to device portability across organisms. In Chapter 3, we
present a ribozyme-based regulatory system that modulates transgenic expression of the proliferative cytokines IL-2 and IL-15, demonstrating drug-responsive, rapid, and reversible T-cell proliferation control in murine cells and animal models. In Chapter 4, we demonstrate the ability to regulate the growth of primary human central memory T cells with drug-responsive ribozyme switch systems. We further present characterization results on the human natural killer cell line NK-92 as a potential model for device development and optimization in human cells. In Chapter 5, we present the development of drug-responsive miRNA switches that regulate the expression of endogenous IL-2 receptor chains and modulate signaling activities in the IL-2/IL-15 pathways. This trans-acting system for endogenous gene expression regulation complements the cis-acting ribozyme-based system and expands the repertoire of RNA-based designs for multi-layered gene expression control in mammalian cells. In Chapter 6, we discuss efforts in selecting novel RNA aptamers to clinically suitable small-molecule targets, including phenobarbital, folic acid, and vitamin B\textsubscript{12}. Various selection and binding characterization methods were developed and evaluated, and the results serve to inform ongoing efforts in aptamer selection.

Synthetic RNA-based devices have broad applications in areas ranging from energy production to metabolic engineering. The work presented here demonstrates the capabilities and potential applications of RNA-based regulatory systems in improving next-generation treatment options for critical diseases. Although much optimization remains possible, this work illustrates one approach to the engineering of biological systems that may be applied to future endeavors in synthetic biological design.
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