Conclusions

It is becoming increasingly evident that RNA is a functionally diverse and versatile molecule that plays an essential role in regulating gene expression in various organisms. Regulation can occur at different levels in the gene expression pathways, including transcription and translation, and through diverse mechanisms, including RNA-RNA base-pairing interactions and cleavage via different physical implementations, *in cis* or *in trans*. RNA is a flexible molecule that can adopt different conformations and fold into secondary and tertiary structures, yet still possesses the ability to undergo dynamic conformational changes, which allow it to interact with a wide range of biological molecules such as DNA, protein, and other RNA molecules. RNA molecules exhibit ligand recognition properties, through which they can sense their environment, and allosteric binding properties, through which they can self-regulate their own activity. These properties enable RNA to function as precise molecular sensors and autonomous control systems that require no additional aid from proteins as intermediate sensor or actuator elements.

Due to its unique array of functional properties, RNA is a powerful platform for the design of regulatory molecules for a wide range of biotechnological and medical applications. Unlike larger biomolecules such as proteins, the functional activity of RNA is more directly defined by its secondary structure. This relationship between RNA secondary structure and function, in combination with predictive RNA secondary structure / energetic folding programs, has enabled molecular engineers to construct diverse synthetic regulatory RNA elements and to manipulate biological processes at the molecular level with greater flexibility and reliability.

RNA engineering has rapidly emerged in the last decade, as RNA has proven to be a versatile molecule capable of performing various cellular functions that go beyond passive transfer of genetic information between the genome and the proteome. RNA represents an attractive and excellent substrate for the construction of gene control devices, and thus numerous synthetic RNA devices have been engineered for regulating the expression of various target genes. Supported by technologies that allow the generation of RNA sensors, researchers have integrated sensory elements and developed many examples of RNA devices comprised of integrated sensor and actuator domains that control gene expression in response to specific target molecules. While these examples have made profound contributions in advancing the field of RNA engineering, they either fail to function *in vivo*, or lack one or more key engineering properties, such as portability, scalability, and modularity. In addition, their artisanal designs and construction do not support component reuse and a general device composition framework.

We set out to develop a functional composition framework that supports the reliable design and construction of synthetic molecular switches and sensors from modular functional components to support the generation of many such devices that can be implemented in a broad range of biotechnological applications such as gene expression regulation, biosensors, therapeutic molecule design, metabolic reprogramming, and tools for elucidating cellular function. The RNA devices are based on a modular, extensible, and portable regulatory platform that enables the tailored construction of application-specific switches and sensors responsive to user-defined target ligands without complex device redesign. In addition, the RNA devices can be utilized in concert

with other gene-regulatory components such as inducible promoters, and synergistic and application-specific gene-regulatory responses can be readily obtained through such combinatorial designs. For instance, similar to the strategies described previously, such as energetic tuning of individual devices or signal integration within the 3' UTR by multi-copy device expression, the regulatory dynamic range of an RNA device can be altered to fit particular applications by expressing the device under the control of an inducible promoter at an appropriate induction level. Such strategies will enable the tuning of the device response levels in the absence and presence of a target ligand to match the application-specific threshold levels. Such component matching between the ribozyme switches and other biological components in the genetic system will broaden the utility of this molecular tool. Furthermore, validation of the preliminary results observed with the p50-responsive ribozyme switches is important to demonstrate the extensibility of the sensing platform to a different class of targets, protein ligands, to extend the utility of this platform. In addition, technological advances that enable the efficient generation of well-characterized libraries of sensor components (RNA aptamers) that recognize biologically-relevant molecules, function in the cellular environment, and are compatible with the described composition frameworks will be critical to the broader implementation of these frameworks for the construction of 'designer' RNA devices capable of processing user-specified environmental and intracellular signals.

The functional performance of the RNA devices described in this thesis can also be improved through the integration of future scientific and technological advances. For instance, advances in RNA engineering that provide insights into RNA structure-function relationships and improved predictions of RNA secondary and tertiary structures relevant

to *in vivo* folding environments will enable the development of RNA devices with more robust functional performance. In addition, modeling tools that can predict both thermodynamic and kinetic properties of RNA folding *in vivo*, incorporate tertiary interactions, and link those properties to functional states and gene expression pathways will support future design tools that efficiently optimize and program device properties *in silico*.

Engineered RNA devices have significant potential to transform our ability to interface with biological systems and to fundamentally change the ways in which we manipulate, program, and probe living systems. Many effective solutions to the societal challenges faced today, including ‘renewable’ energy and the ‘green’ environment, sustainability, and health and medicine, will be derived from engineered biological systems, and thus we hope that our engineered RNA devices play a role in achieving these solutions.