# Chapter 4

# Conclusions

The emerging field of synthetic biology has produced a vast array of engineered molecular devices, enabling investigation of cellular function and programmed control of new phenotypic behaviors in biological systems[1–4]. These devices are generally composed of protein or RNA, two biological macromolecules whose sequence determines their three-dimensional shape, dictating their ability to bind to other molecules and catalyze chemical reactions. RNA and protein engineering have greatly expanded the capabilities of these macromolecules, enabling functions not found in natural biological systems[3,5–8].

Synthetic molecular devices have been used to regulate gene expression in a wide variety of organisms, from prokaryotes to microbial eukaryotes to humans[9–11]. Some of these genetic control platforms are able to process molecular input into increases or decreases in gene expression output by combining a sensor component with an actuator component[1,12]. Such platforms exhibit the greatest utility when the components are easy to design and optimize, and when different components can be integrated together in predictable ways without disrupting their individual functions.

RNA is particularly well suited as a substrate for the implementation of molecular gene-regulatory devices. RNA molecules can hybridize with RNA and DNA through base-pairing interactions, and bind to small molecules and proteins by adopting specific conformations[13,14]. They are also able to catalyze various chemical reactions, including the lysis of phosphodiester bonds[15,16]. The binding and catalytic functions of RNA strands are largely dictated by their secondary structure, which can be predicted by computational models of RNA folding[17–19]. In contrast, protein function largely depends on complex tertiary interactions, which are currently far more challenging to predict from

the primary sequence alone. Furthermore, the ability of RNA to be replicated by reverse transcription and PCR enables the facile *in vitro* selection of RNA molecules with novel functions from large libraries of different sequences[20,21].

The ability of RNA enzymes to cleave phosphodiester bonds is exploited in the engineering of the ribozyme switch platform, in which cleavage of an mRNA strand by a hammerhead ribozyme causes silencing of the encoded gene in response to ligand binding to an aptamer[12]. With the aid of structure prediction software, ribozyme switches were designed to adopt distinct cleavage-active and cleavage-inactive conformations, with ligand binding stabilizing the conformation in which the aptamer sensor component is properly formed[12]. Both ON and OFF switches were demonstrated to regulate gene expression in yeast and mammalian cells and, importantly, replacement of the aptamer component to sense an alternate ligand did not require extensive redesign of the device[12,22,23]. However, the platform was limited to the regulation of transgenes in response to small molecule inputs.

We attempted to extend the capabilities of the ribozyme switch platform to two new functions: the regulation of endogenous genes and the sensing of protein inputs. We were unable to demonstrate ribozyme activity in trans, and the limitations we discovered suggest that this platform is not as promising as other trans-acting platforms such as those based on RNAi[24–26] and CRISPRi[27]. We were successful, however, in developing novel protein-responsive ribozyme switches for regulating genes in cis in human cells. We demonstrated a higher level of ligand-responsiveness than previously described small-molecule-responsive ribozyme switches in mammalian systems, and we showed that cytoplasmic and nuclear localization of ligand were each sufficient to elicit switching

activity. We also demonstrated the versatility of our switch platform with a ribozyme switch responsive to an alternative protein ligand.

In our attempts to develop new devices responsive to various protein ligands, we found that integration of aptamers into the platform is a challenging process. Further study is needed in order to extend our platform to diverse ligands, making the process of generating new devices more reliable and straightforward. We rationally designed each device presented here, but in the future a wider sequence space could be explored using high-throughput *in vivo* screening methods[20,28,29] to assay large libraries of randomized devices. As new sensor components are generated by *in vitro* selection, we hope that improved screening strategies will enable them to be integrated into our switch platform.

Our ribozyme switch is able to respond to proteins in either the nucleus or the cytoplasm, while previously described mammalian gene-regulatory devices have required specific localization of ligand in order to produce a switching response. Our platform is therefore unable to detect changes in protein distribution across subcellular compartments, but it is more versatile than previous platforms in that ligand input choice is not restricted to proteins localized to just one compartment.

We developed a device responsive to β-catenin, a signaling protein with an important role in cancer[30]. This device and other ribozyme switches that respond to disease markers could be used to noninvasively detect diseased cellular states. Furthermore, such switches could be used to control cell fate by, for example, regulating the expression of a proapoptotic transgene. In this way a genetically encoded therapeutic effect could be targeted to diseased cells while leaving healthy cells unaffected. Coupling our device with other synthetic biology components such as positive feedback

or amplifier systems could expand the dynamic range of switch response and enable tuning of activity to match application-specific phenotypic thresholds. As the field of synthetic biology continues to advance, we hope the molecular device platform we have developed will be a useful tool for protein-responsive gene regulation.

## References


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