# Cell-Targeted Regulation of Gene Expression through Synthetic RNA Devices

Thesis by

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## Abstract

The ability to interface with and program cellular function remains a challenging research frontier in biotechnology. Although the emerging field of synthetic biology has recently generated a variety of gene-regulatory strategies based on synthetic RNA molecules, few strategies exist through which to control such regulatory effects in response to specific exogenous or endogenous molecular signals. Here, we present the development of an engineered RNA-based device platform to detect and act on endogenous protein signals, linking these signals to the regulation of genes and thus cellular function.

We describe efforts to develop an RNA-based device framework for regulating endogenous genes in human cells. Previously developed RNA control devices have demonstrated programmable ligand-responsive genetic regulation in diverse cell types, and we attempted to adapt this class of cis-acting control elements to function in trans. We divided the device into two strands that reconstitute activity upon hybridization. Device function was optimized using an *in vivo* model system, and we found that device sequence is not as flexible as previously reported. After verifying the *in vitro* activity of our optimized design, we attempted to establish gene regulation in a human cell line using additional elements to direct device stability, structure, and localization. The significant limitations of our platform prevented endogenous gene regulation.

We next describe the development of a protein-responsive RNA-based regulatory platform. Employing various design strategies, we demonstrated functional devices that both up- and downregulate gene expression in response to a heterologous protein in a human cell line. The activity of our platform exceeded that of a similar, small-molecule-

responsive platform. We demonstrated the ability of our devices to respond to both cytoplasmic- and nuclear-localized protein, providing insight into the mechanism of action and distinguishing our platform from previously described devices with more restrictive ligand localization requirements. Finally, we demonstrated the versatility of our device platform by developing a regulatory device that responds to an endogenous signaling protein.

The foundational tool we present here possesses unique advantages over previously described RNA-based gene-regulatory platforms. This genetically encoded technology may find future applications in the development of more effective diagnostic tools and targeted molecular therapy strategies.

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