Engineering Input-Responsive RNA Devices for Dynamic Cellular Control

Anthony M. Stohr
Advisors: Dr. Mark A. Blenner and Dr. Wilfred Chen
Committee Members: Dr. Aditya M. Kunjapur, Dr. Jeffery Mugridge, and Dr. Kevin V. Solomon

Monday, September 14, 2026, at 9:00 AM (EDT)
366 Colburn Laboratory | https://udel.zoom.us/j/91333151216 | Password: RNA!

Bioengineering has enabled the reprogramming of life by endowing cells with novel and expanded functionalities. For instance, cells have been repurposed as living factories for sustainable chemical production or therapeutic biomanufacturing. Concurrently, advancements in gene-editing technologies enable targeted cellular manipulations to treat a myriad of pathologies. Consequently, precise control over cellular behavior has broad utility across biotechnology and medicine. However, the majority of cellular engineering approaches are static, limiting overall performance by imposing a heavy burden on host cells and preventing dynamic adaptation.

Dynamic regulation mitigates these challenges by allowing engineered cells to continuously sense and respond to their environment. Implementing dynamic regulatory strategies requires repurposing biological components into sensors to detect relevant inputs and actuators to transduce these signals to drive cellular behavior. RNA directly interfaces with the majority of cellular processes, exhibits programmable base-pairing rules, and binds diverse biological targets (DNA, proteins, and small molecules). These features position RNA as an incredibly useful building block for engineering responsiveness yet RNA engineering is in its infancy compared to DNA- and protein-based strategies. In this dissertation, we exploit these advantageous properties to engineer input-responsive RNA devices for dynamic cellular control.

For our first RNA-based regulatory scheme, we engineered metabolite-responsive scaffold RNAs (MR-scRNAs) to develop dynamic CRISPR transcriptional programs. By embedding a molecular switch into a guide RNA scaffold, we were able to conditionally recruit effector proteins to the CRISPR complex enabling input-responsive gene activation. We demonstrated that our MR-

scRNAs are highly specific to the target ligand and exhibit dose-dependent behavior with favorable kinetics and reversibility. We validate the modularity of this strategy by regulating the gene expression of three distinct host organisms across different genetic targets and in response to multiple inputs. We then demonstrated the utility of our device for metabolic engineering applications by using the MR-scRNAs to regulate enzyme expression in response to substrate concentration which enabled balanced pathway expression and improved biochemical production.

We harnessed aptamers to create another regulatory strategy for the control of *trans*-acting devices. To do so, we rationally split RNA aptamers to create RNA fragments whose reconstitution is driven by ligand-binding. We employed these split aptamers to control RNA intron reconstitution thus creating SPLICER, a ligand-responsive *trans*-splicing platform. We established stem engineering as a method to tune split aptamer reconstitution efficiency and applied these findings to develop novel split aptamers that respond to both small-molecule and protein ligands. We deployed SPLICER to regulate the expression of multiple coding and non-coding RNAs. Altogether, we demonstrate two distinct novel RNA-based regulatory strategies that expand the synthetic biology toolbox for conditional cellular control.