RiboTALes: A Novel Paradigm for Transcriptional Control

Abstract
The ability to control the expression of specific genes with molecular effectors is a critical function for the construction of genetic devices. While the Registry of Standard Biological Parts contains a large catalog of inducible promoters, very few of these are actually used in new synthetic devices and many go unused because of poor characterization or effectors' incompatibility. To increase the versatility of existing and novel expression control systems, we propose a new part type that couples transcription activator-like effectors (TALES) with inducible riboswitches. TALEs are proteins secreted by the bacterial pathogen Xanthomonas that contain engineered, sequence-specific DNA binding domains and can act as transcriptional repressors or activators. We regulated TALE activity during translation by pairing TALEs with riboswitches to create RiboTALes. We use RiboTALes to expand the existing library of inducible repression systems. In addition, we show that tuning parameters of RiboTALe increases their functional utility.

Project Motivation
We want to increase the spectrum of possible effectors that can be used to regulate the expression of specific genes.

IT MUST BE EASY TO ENGINEER NEW TRANSCRIPTIONAL REGULATORS THAT SIMULTANEOUSLY TARGET SPECIFIC DNA SEQUENCES AND BIND SPECIFIC EFFECTORS

Solution
Riboswitches are RNA regulatory structures that regulate the initiation of translation changing conformation in the presence of a specific ligand [1]. Transcription activator-like effectors (TALES) are proteins that contain sequence specific DNA binding domains and can act as transcriptional repressors or activators [2]. The DNA binding domains are sequence specific, and can be engineered to bind to any DNA sequence of interest, following now well-understood rules for TALE-DNA binding [3,4]. The combination of these two components allows for a flexible architecture in transcription factors that can be used in any chassis.

Modeling
This model provides a mathematical basis that supports the functionality of our RiboTALe devices and displays the range of responses achievable by our system. By fitting experimental data to the model, we were able to predict the effect of theophylline on system output at a certain arabinose concentration (0.01% in the image below).

This model could be improved by taking into account the effects of context dependence on promoter activity, riboswitch leakage, and the possibility of altered TALE behavior at high concentrations.

Human Practices
Given the complexity of our characterization data we thought it would be appropriate to share our raw data. We realized that openly sharing raw characterization data for all parts in the Registry would be generally good for the synthetic biology community. A successful method for sharing data would fundamentally change the way we interact. We took on that challenge as our main human practices component to our project.

We sought input from JBEI's Hector Garcia-Martin, who gave insight on the design and use-cases of a biological data system, and UC Davis' Bertram Ludäscher, who gave advice on the physical storage of data. Beyond this, sharing data as we interact, the clear solution was to build an online database that allows IGem teams to upload and retrieve raw RiboTALe characterization data. We like to call this database The Depot.

Conclusion
RiboTALes provide a new precedent for transcription factors in synthetic biology. By having a flexible architecture, a modular input, and the ability to be used in any chassis, we believe that RiboTALes can be valuable building blocks for future iGEM teams.

We believe the Depot provides a better foundation for Biobrick characterization. In the spirit of iGEM and academia, we believe the database can promote collaboration, innovation, and sharing of raw characterization data.

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References