RiboTALs: A Novel Paradigm for Transcriptional Control

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Abstract

Despite the fact that the Registry of Standard Biological Parts contains a large number of inducible promoters, the actual usage of these parts is limited by a very small number. In order to increase the versatility of expression control systems, we propose a new system coupling transcription activator-like effectors (TALEs) with inducible riboswitches. TALEs are proteins secreted by the bacterial pathogen Xanthomonas that contain engineerable, sequence-specific DNA binding domains, and can act as transcriptional repressors or activators. We plan to manipulate TALE activity by subjecting them to inducers through riboswitches and promoters. By pairing TALEs with riboswitches, we can expand the existing library of inducible expression systems. In addition, we hope to modify the parameters of our system to show the tunability and modularity of our overall construct. Through proper characterization, we believe that IGEM teams may also use these modular repression devices for the future.

Project Motivation

The registry grows in size every year, but only a few parts are being reused for detecting inputs.

We need more available inputs for transcriptional and translational control.

Solution

RiboTALs are RNA regulatory structures that in the presence of a specific ligand change conformation to regulate the initiation of translation. Transcription activator-like effectors (TALEs) are proteins that contain sequence-specific DNA binding domains and can act as transcriptional repressors or activators. The DNA binding domains are sequence specific, and can be engineered to bind to any DNA sequence of interest. Following how well-understood rules for TAL-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 RiboTALs device, and shows the wide variety of system responses achievable through the modulation of the engineerable and tunable elements of our construct. The equations here model concentrations of transcription factors, the probability of active complexes (unbound promoters and TALE binding sites, bound riboswitch), and the dynamics of the system itself.

RiboTALe Characterization

This image shows RiboTAL mediated repression of gene expression directly correlated with increasing thiopephrine concentration, indicating that our system is functional, tunable. Maximum repression was 2.6 fold.

Two thiopephrine-responsive riboswitches were tested that exhibited tightly controlled and leaky induction [6]. [7] The consistent difference in system behavior indicates that riboswitch leakiness can function as an engineerable source of tunability.

The Depot

From the registry to the wiki, IGEM is founded upon the idea of sharing. Having an open culture allows scientific communities to validate successes and understand failures, while supporting future researchers downstream. However, as we searched through the registry, we realized that teams focused on sharing highly processed versions of their data, but rarely found the time to share their raw numbers, making independent evaluation of the experiment challenging. Sharing data for any part type in a way that is useful to everyone is an extremely difficult objective, but we wanted to take a step towards making it easier. Now we are pleased to introduce our step towards that solution. The Depot, an open Biobrick characterization database to further promote sharing in iGEM.

We collaborated with Stanford-Brown, UCSF, ZJU-China, and Bioleited-Germany to initially populate the database with data from the Anderson Family of Promoters. We also provided raw data on the parts that we created this summer.

Other Efforts in Human Outreach:

- Provided lab materials for the biotechnology class at Davis Senior High School
- Worked with classmate Nicholas Armstrong to create a Synthetic Biology Club at UC Davis

Conclusion

RiboTALs 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 RiboTALs can be valuable Biobricks 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


The image shows a graph with labeled axes and a legend for the data points. The graph is labeled "RiboTAL mediated repression," and the y-axis represents concentration, while the x-axis represents time. The legend includes symbols for different conditions, such as "no repression," "repression," and "expression." The data points are scattered across the graph, indicating the differential expression levels under various conditions.