Synthetic Transcription Factor Cooperativity Analysis Using ODE Polymerase Model

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Abstract

Synthetic Transcription Factors (STFs) designed by the Collins Lab have shown an unusually strong ability to amplify gene expression by simply adding tandem operators. The Polymerase Model was used to analyze this system in hopes to understand the cooperativity values associated with such large effects. The result of this procedure was a rational expression that exhibited strange and not completely understood implications on the cooperativity values we sought. Although our model's analysis is not finished, it seems to be hinting at ample amounts of restrictions and very large higher-order cooperativity values that should be dissected further to understand their mechanisms and possible implications in designing STFs in the future.

Background

In the Collins Lab's publication, "A Synthetic Biology Framework for Programming Synthetic Eukaryotic Transcription Factors," an interesting observation was made. The transcriptional outputs of STFs with different numbers of tandem operators were measured, and there was a vast difference in output depending on the operator count. In particular, the STF with two distinct operators had 14x the transcriptional output of the STF with only one. Meanwhile, a similar system that utilized two different STFs (each with a single operator) had an increase in output of only 1.5x. The pronounced difference in output due to operator count in is rarely observed in natural contexts and warranted further attention.

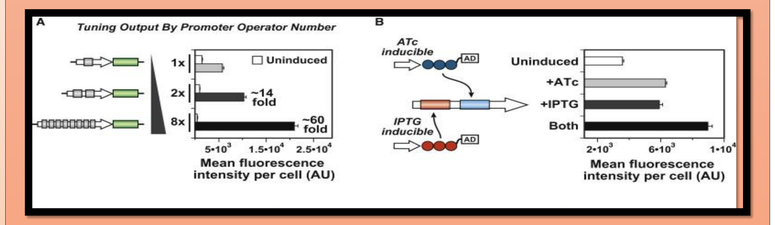

Figure 1: sTF Transcriptional Output data from Collins's paper

Linear Framework

The linear framework, developed by the Gunawardena Lab, offers a robust basis for analysis of gene regulation on the single-gene level. Whether it's used in simple prokaryotic systems or more complex eukaryotic systems, the linear framework has been indispensable in recent years for quantitative analysis of genetic regulation.[2] One of its various extensions, the Polymerase Model, predicts transcriptional output based on the binding states of transcription factors and RNA Polymerase. Thus, the Polymerase Model can be used to analyze the output of STFs observed by the Collins Lab.

Polymerase Model

The Polymerase Model takes the form of an ordinary differential equation describing the rate of change in a gene's mRNA product concentration, effectively showing how heavily a gene is being expressed at steady-state. This equation is based on the probabilities of the various possible microstates for a gene of interest. Two unique stipulations in the Polymerase Model are that only the microstates with RNA Polymerase bound are responsible for the production of mRNA and RNA Polymerase cannot be bound without at least one transcription factor preceding it. Each microstate's probability is calculated with concentrations (x,y, and z), cooperativities (Ω and ω's), and binding affinities (K's).

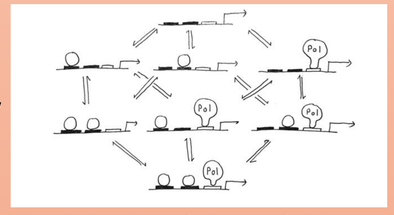

Figure 2: Example set of microstates for Polymerase Model [3]

STF Polymerase Models

When applied to the system of interest, the Polymerase model was used twice. First to make an equation for the STF with only 1 operator, then for the STF with 2. These equations put the expression level of the gene (or E1 and E2) in terms of the individual microstates' probabilities.

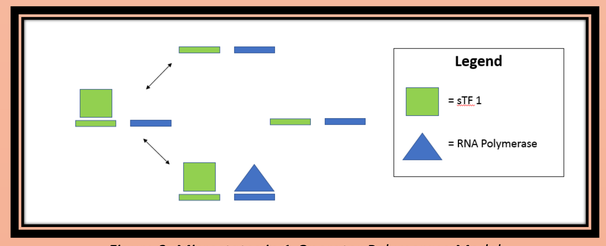

Figure 3: Microstates in 1-Operator Polymerase Model

Due to there being multiple elements that potentially have unique cooperative behaviors, a higher-order cooperativity (Ω) is introduced into the equation for the 2-operator model that wasn't present in the 1-operator model.

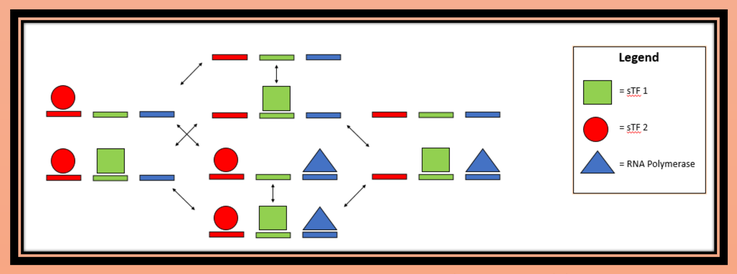

Figure 4: Microstates in 2-Operator Polymerase Model

1-Operator vs. 2 Operator Model Comparison Results

The concentrations of the STFs are at saturation in the system of interest, so the limit of these equations as the STF concentrations approach infinity are taken. These limits are what were compared. After setting the limit of E2 equal to 14 times the limit of E1 and simplifying, the resulting equation is:

$$\Omega = \frac{14\omega_{12}}{1 - 13K_3 z}$$

This result is a rational expression but it has strange behaviors and implications due to its denominator's inability to equal 0 and the fact that cooperativities cannot be negative.

Discussion

Analysis of the resulting cooperativity equation has not yet been fully completed, but our initial outlook on it is that there are several important restrictions necessary to allow it to be valid. Since the denominator in the equation cannot be 0, there is an asymptote restricting the range of K_3 and z values. Additionally, cooperativities cannot equal 0, as they are defined to be greater than 0. A 'negative' cooperativity is actually just below 1. To ensure both sides of the equation have the same sign, and can thus both be positive, the range of K_3 and z values is further restricted to values that allow the product of K_3 and z to be smaller than $\frac{1}{13}$.

After having met these conditions though, one side of the equation is still multiplied by 14, meaning the higher order cooperativity has to be very high in comparison to a normal cooperativity. Cooperativities as high as those suspected from these STFs are exceedingly rare in natural environments. They are of considerable interest to geneticists as their mechanisms could hold valuable insights into previously unearthed genetic regulatory effects. Thus, further study into the mechanisms by which this cooperativity arose are recommended and encouraged.

References

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2. Gunawardena, J. (2012). A Linear Framework for Time-Scale Separation in Nonlinear Biochemical Systems. PLoS One *7*.
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