A mathematical model for the use of riboswitches-like elements in transcriptional regulation in *E. coli*

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1 Introduction

According to the 2017 SVCE Chennai iGEM team,

"While transcriptional regulators are composable and can be easily used to regulate the function of complex genetic circuits, they are difficult to engineer as their mechanism involves action on moving RNA polymerase and hence ill-defined kinetic and dynamic structural factors play a major role. On the other hand translational regulators can be easily engineered using predictive thermodynamic and kinetic models but are constrained to act on single genes and cannot be assembled into complex genetic circuits as translation initiation at RBS is a distributive process."

Combining the ease of construction, characterization and realization of a translational regulatory element with the modularity of the transcriptional regulator, the 2017 SVCE Chennai iGEM team set out to architect a genetic circuit containing a *riboswitch* governing the fate of the transcriptional regulator tnaC. [1]

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2 The System

The *Tryptophanase* (tna) operon in *E. coli* consists of transcribed leader regulatory region, containing a coding region for the 24-residue leader peptide *TnaC*. The leader region is promptly followed by two structural genes downstream, *tnaA* and *tnaB* that encode tryptophanase and tryptophan permease. Tryptophanase is a catabolic enzyme that degrades tryptophan to, among other things, pyruvate and ammonia, allowing *E. coli* to use tryptophan as a carbon and nitrogen source. The initiation of the transcription is regulated through typical catabolite repression. At low concentrations of tryptophan, rho-dependent termination of the operon transcription occurs immediately after the *tnaC* at a transcriptional pause site. [3]

Tryptophan induction does however require the synthesis of TnaC. This is generally facilitated by inhibitory action of the tryptophan on the release factor 2 that would have terminated the translation. The nascent polypeptide product remains uncleaved, its retention stalling the ribosome at the stop-codon, which subsequently prohibits rho-factor association. Thus, elongation occurs. The polypeptide action will be modeled as a transcription factor (further clarification of underlying assumption can be found in section 3). [4]

If the native, constitutive RBS of the tna operon were switched to an engineering RBS with an inducible ON/OFF-switch, transcription of the TnaC and subsequent induction of downstream genes would only transpire in concurrence with the ON-mode of the RBS. Thus, through clever design of the RBS, the *tnaC* can be used as an adaptor between transcriptional and translational regulation of gene expression.

The two inducible translational regulatory elements that will be considered is the pH-dependent riboswitch and the riboswitch-like module RNA-thermometer (RNAt). Both units exploit the secondary structure that is generated by base-pair association within the mRNA.

The RNA thermometer are usually located in the 5'UTR of the mRNA and consists of a secondary structure involving the Shine-Dalgarno sequence or the AUG start codon. That is to say, the RBS or start codon is thermo-dependently sequestered. RNAts have proven to serve as functional components in synthetic biology and related fields due to their regulatory versatility; the ability to melt and re-form the secondary structure offers rare bidirectionality in transcriptional regulation. [5]

The mechanism of the pH-dependent riboswitch resembles that of the RNAt to the extent that any induced regulatory bias occurs not as a consequence of ligand asso-

ciation but rather as a response to the physicochemical conditions of the cell. Under alkaline conditions, increased longevity of RNAp pauses at (multiple) well-defined pause sites leads to a different structural formation of the secondary mRNA structure around the Shine-Dalgarno sequence or the AUG codon, allowing previously blocked translation. [2]

3 Model

3.1 Model overview

The following scheme was provided by the SVCE Chennai iGEM team as a visual representation of the intended genetic circuit.

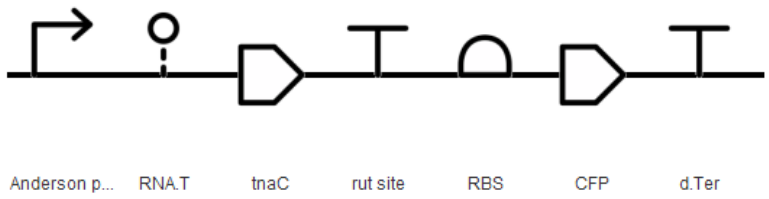

Figure 1: Visualization of the intended gene sequence of the adaptor [1]

As apparent from the construction, the translational regulation, that is, the RBS modification will be realized by a riboswitch. More specifically, a RNA thermometer and a pH-responsive riboswitch will be used.

The mathematical analysis of the genetic circuit will be focused on chemical dynamics of the system and will be derived in accordance with the law of mass action. Upon request, only deterministic chemical reaction pathways will be considered.

3.2 Model derivation

We proceed by modeling a riboswitch that operates via translational repression. Through constitutive expression of the mRNA transcript, we assume two conformational states M_A and M_B, corresponding to the ON/OFF behavior of the riboswitch,

transcribed in proportionate amounts from the same promoter O_M. The transcription concerns that of genes upstreams that of the *TnaC*.

$$\mathrm{O_M} \xrightarrow{\mathrm{k_{tA}}} \mathrm{O_M} + \mathrm{M_A} \tag{1}$$

$$\mathrm{O_M} \xrightarrow{\mathrm{k_{tB}}} \mathrm{O_M} + \mathrm{M_B} \tag{2}$$

It is assumed that the transcripts can be interconverted between the two conformational states via first order reaction.

$$\mathrm{M_A} \xrightarrow{\mathrm{k_{a1}}} \mathrm{M_B} \tag{3}$$

$$\mathrm{M_B} \xrightarrow{\mathrm{k_{d1}}} \mathrm{M_A} \tag{4}$$

The transcripts are subsequently translated into the polypeptide TnaC, T via first order catalyzed reactions.

$$\mathrm{M_A} \xrightarrow{\mathrm{k_{pA}}} \mathrm{M_A} + \mathrm{T} \tag{5}$$

$$\mathrm{M_B} \xrightarrow{\mathrm{k_{pB}}} \mathrm{M_B} + \mathrm{T} \tag{6}$$

The translated polypeptide T will be considered as a transcription factor using the following logical basis. mRNA Elongation will occur if TnaC acts upon the ribosome by stalling it over the terminator site (leakage is disregarded), thus preventing rho-dependent termination. Conceptually, this corresponds to the positive action of

a molecule on the initiation of transcription. This resembles the action of an activator (positive transcription factor) on an enhancer sequence. Ergo, the TnaC will henceforth be considered a transcription factor and modeled as such.

The polypeptide T associate with the DNA-bound $RNAp$ to generate the active $RNAp^{\star}$, consequently inducing lag and transcription of downstreams genes.

$$\mathrm{RNAp} + \mathrm{T} \xrightarrow{\mathrm{k_{a2}}} \mathrm{RNAp}^{\star} \tag{7}$$

$$\mathrm{RNAp}^{\star} \xrightarrow{\mathrm{k_{d2}}} \mathrm{RNAp} + \mathrm{T} \tag{8}$$

The transcription is modeled as a conventional first order catalyzed reaction to yield the mRNA elongation M which is translated into a protein P via similar kinetics.

$$\mathrm{RNAp}^{\star} \xrightarrow{\mathrm{k_{tT}}} \mathrm{RNAp}^{\star} + \mathrm{M} \tag{9}$$

$$\mathrm{M} \xrightarrow{\mathrm{k_{P}}} \mathrm{M} + \mathrm{P} \tag{10}$$

Degradation is assumed to proceed through first order reactions.

$$\mathrm{M_A} \xrightarrow{\mathrm{k_{dMA}}} \emptyset \tag{11}$$

$$\mathrm{M_B} \xrightarrow{\mathrm{k_{dMB}}} \emptyset \tag{12}$$

$$\mathrm{M} \xrightarrow{\mathrm{k_{dM}}} \emptyset \tag{13}$$

$$\mathrm{T} \xrightarrow{\mathrm{k_{dT}}} \emptyset \tag{14}$$

$$\mathrm{P} \xrightarrow{\mathrm{k_{dP}}} \emptyset \tag{15}$$

Table 1: Model species

Notation	Description
$\mathrm{O_M}$	Constitutive promoter
$\mathrm{M_A}$	Riboswitch conformation A
$\mathrm{M_B}$	Riboswitch conformation B
T	TnaC transcription factor
RNAp	DNA-bound RNAp
$\mathrm{RNAp}^\star$	Active DNA-bound RNAp
M	Generic mRNA
P	Generic protein

3.3 Deterministic equations

We proceed to derive the deterministic equations that govern the proposed dynamics. As usual, the reaction is assumed to take place in a homogeneous, well stirred and constant volume compartment. Using the law of mass action, the reaction rates ν_i for reactions 1 to 15 are constructed.

$$\nu_1 = \mathrm{k_{tA}} \tag{16}$$

$$\nu_2 = \mathrm{k_{tB}} \tag{17}$$

$$\nu_3 = \mathrm{k_{a1}} \cdot [\mathrm{M_A}] \tag{18}$$

$$\nu_4 = \mathrm{k_{d1}} \cdot [\mathrm{M_B}] \tag{19}$$

$$\nu_5 = \mathrm{k_{pA}} \cdot [\mathrm{M_A}] \tag{20}$$

$$\nu_6 = \mathrm{k_{pB}} \cdot [\mathrm{M_B}] \tag{21}$$

$$\nu_7 = \mathrm{k_{a2}} \cdot [\mathrm{RNAp}] \cdot [\mathrm{T}] \tag{22}$$

$$\nu_8 = \mathrm{k_{d2}} \cdot [\mathrm{RNAp^\star}] \tag{23}$$

$$\nu_9 = \mathrm{k_{tT}} \cdot [\mathrm{RNAp^\star}] \tag{24}$$

$$\nu_{10} = \mathrm{k_p} \cdot [\mathrm{M}] \tag{25}$$

$$\nu_{11} = \mathrm{k_{dmA}} \cdot [\mathrm{M_A}] \tag{26}$$

$$\nu_{12} = \mathrm{k_{dmB}} \cdot [\mathrm{M_B}] \tag{27}$$

$$\nu_{13} = \mathrm{k_{dM}} \cdot [\mathrm{M}] \tag{28}$$

$$\nu_{14} = \mathrm{k_{dT}} \cdot [\mathrm{T}] \tag{29}$$

$$\nu_{15} = \mathrm{k_{dP}} \cdot [\mathrm{P}] \tag{30}$$

The system of ordinary differential equations is constructed by balancing each model specie with respect to the reaction rates.

$$\frac{d}{dt}[\mathrm{O_M}] = 0 \tag{31}$$

$$\frac{d}{dt}[\mathrm{M_A}] = \nu_1 - \nu_3 + \nu_4 - \nu_{11} \tag{32}$$

$$\frac{d}{dt}[\mathrm{M_B}] = \nu_2 + \nu_3 - \nu_4 - \nu_{12} \tag{33}$$

$$\frac{d}{dt}[\mathrm{T}] = \nu_5 + \nu_6 - \nu_7 + \nu_8 - \nu_{14} \tag{34}$$

$$\frac{d}{dt}[\mathrm{RNAp}] = -\nu_7 + \nu_8 \tag{35}$$

$$\frac{d}{dt}[\mathrm{RNAp}^{\star}] = \nu_7 - \nu_8 \tag{36}$$

$$\frac{d}{dt}[\mathrm{M}] = \nu_9 - \nu_{13} \tag{37}$$

$$\frac{d}{dt}[\mathrm{P}] = \nu_{10} - \nu_{15} \tag{38}$$

Table 2: Model rate constants.

Notation	Unit	Description
k_{tA}	s^{-1}	Transcriptional rate constant M_A
k_{tB}	s^{-1}	Transcriptional rate constant M_B
k_{t1}	s^{-1}	Conformational change rate constant M_A
k_{d1}	s^{-1}	Conformational change rate constant M_A
k_{pA}	s^{-1}	Translational rate constant M_A
k_{a2}	$M^{-1} \cdot s^{-1}$	Translational rate constant M_A
k_{d2}	s^{-1}	RNAp + T association rate constant
k_{tT}	s^{-1}	$RNAp^{\star}$ dissociation rate constant
k_P	s^{-1}	Transcriptional rate constant M
k_{dMA}	s^{-1}	Translational rate constant M_A
k_{dMB}	s^{-1}	Degradation rate constant M_A
k_{dM}	s^{-1}	Degradation rate constant M_B
k_{dT}	s^{-1}	Degradation rate constant M
k_{dP}	s^{-1}	Degradation rate constant T
P	s^{-1}	Degradation rate constant P

3.4 Temperature dependency

The defined system can be turned into a RNA thermometer by adding a temperature dependency on every rate constant k_i. This will then take account for the major change in the systems behavior caused by a shift in temperature. However, since it is generally hard to let alone estimate parameters for a given stationary temperature,

it might be sufficient to only describe those parameters that actually governs the switching mechanism itself. For this system, those parameters are k_{ai}, k_{d1}, k_{tA} and k_{tB} which govern the equilibrium constant between M_A and M_B. As an example, assuming M_B is translated at a higher speed M_A than but present in a smaller amount at lower temperatures, an increase in temperature will increase the relative amount of M_B and thus the total translation speed. Consequently, a temperature regulated translational riboswitch is obtained. The temperature dependency can be added by fitting the parameters to the Eyring equation:

$$\mathrm{k_i} = \frac{\mathrm{K_B T}}{\mathrm{h}} \cdot \mathrm{e}^{\frac{\Delta \mathrm{G}^{\ddagger}}{\mathrm{RT}}} \tag{39}$$

where $k_B$is the Boltzmann's constant, T temperature, h Planck's constant, $\Delta G^{\ddagger}$ Gibbs energy of activation and R the ideal gas constant.

References

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