Nonlinear Dynamic Model of Escherichia coli Thiamine Pyrophosphate Riboswitch

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ABSTRACT: In this paper, we investigate the nonlinear dynamic behavior of TPP (thiamine pyrophosphate) riboswitches in *E. coli* (*Escherichia coli*). TPP riboswitches are highly conserved RNA regulatory elements, embedded within the 5' untranslated region of three TPP biosynthesis operons. The three operons *thiCEFSGH*, *thiMD*, and *thiBPQ* are involved in the biosynthesis, salvage, and transport of TPP, respectively. TPP riboswitches modulate their expressions in response to changing TPP concentration, *without* involving protein cofactors. Interestingly, the expression of *thiMD* is regulated at the translational level, while that of *thiCEFSGH* at both levels of transcription and translation. We develop a mathematical model of the TPP riboswitch's regulatory system possessed by *thiCEFSGH* and *thiMD*, so as to simulate the time-course experiments of TPP biosynthesis in *E. coli*. The simulation results are validated against three sets of reported experimental data in order to gain insight into the nature of steady states and the stability of TPP riboswitches, and to explain the biological significance of regulating at level of transcription or translation, or even both. Our findings suggest that in the TPP biosynthesis pathway of *E. coli*, the biological effect of down-regulating *thiCEFSGH* operon at the translational level by TPP riboswitch is less prominent than that at the transcriptional level.

Keywords: riboswitches, RNA, gene regulation, nonlinear, deterministic.

Abbreviations: mRNA, messenger RNA; TPP, Thiamine Pyrophosphate; *E. coli*, *Escherichia coli*;

1 INTRODUCTION

Precise genetic control is an essential survival feature of living systems, as cells must respond to a multitude of biochemical signals and environmental cues by varying genetic expression patterns. Introduced in the early 1960s, the operon concept [1] was postulated that all protein-coding transcriptional units are controlled by means of operons subject to mechanisms of genetic control. Presumably, such mechanisms always involve protein factors that could sense chemical or physical stimuli, and then modulate the expression of corresponding gene(s) by selectively interacting with the relevant DNA or mRNA (messenger RNA) sequence.

Protein factors typically bind to DNA in order to modulate gene expression through various mechanisms [2-6] such as transcription initiation (e.g. *E. coli lacI* [1] and *trpR* [7] repressors regulate *lac* and *trp* biosynthesis operons, respectively by binding to their corresponding operator and blocking them from the RNA polymerase), or by binding to mRNA to control termination at either level of transcription (e.g. *B. subtilis PyrR* repressor regulates *pyr* biosynthesis operon [8]) or of translation (e.g. *B. subtilis trp RNA-binding attenuation protein* regulates *trp* biosynthesis operon [9]). Among them, mathematical models analyzing the nonlinear dynamic behavior of *E. coli lac* [10] and *trp* [11;12] operons, are the most comprehensively investigated.

Although proteins fulfill most requirements that biology has for enzyme, receptor, and structural functions, it has only been discovered in early 2002 that certain natural mRNAs known as "Riboswitches" [13-20] can also serve in these capacities. This unexpected discovery defies the central dogma that DNA acts *purely* as a storage of information, RNA is *solely* the intermediate, and protein performs as the vehicle for catalytic reactions. Riboswitches are metabolite-responsive RNA regulatory elements, typically embedded within the 5' untranslated region before the protein-coding mRNAs to be expressed. They modulate the expression of adjacent gene(s) or operon(s) in response to changing concentrations of metabolites, *without* involving protein cofactors.

In Figure 1, the schematic representation of riboswitch comprises two functional and distinct structural domains, namely the "aptamer" and the "expression platform". The former serves as a natural aptamer that binds selectively to the target metabolite, while discriminates other organic molecules with both high specificity and affinity. Riboswitch must first be activated via the binding of a metabolite molecule to the aptamer domain. The formation of aptamer-metabolite complex induces allosterically structural conformation in the expression platform, which in turn modulates expression of the adjacent gene(s) or operon(s) at either level of transcription or translation. At the transcriptional level, the aptamer-metabolite complex induces the formation of an "intrinsic terminator" in the expression platform. This stem-loop structure causes the RNA polymerase to abort transcription prematurely before the protein-coding mRNA has been synthesized. At the translational level, the aptamer-metabolite complex triggers the formation of a "sequestor" secondary structure in the expression platform to occlude the ribosome-binding site, thus preventing translation initiation.

Diverse molecular regulatory mechanisms by riboswitches have been ubiquitously identified in genomes of bacteria, archaea, and even eukarya. Several classes of riboswitches distinguished by the types of metabolites that they bind to, have been characterized. These target metabolites include the nucleobases guanine [21] and adenine [22], the amino acid lysine [23;24], as well as the coenzymes TPP (thiamine pyrophosphate) [25-29], FMN (flavin mononucleotide)

[27;30], SAM (S-adenosylmethionine) [31-34], B_{12} (adenosylcobalamin) [35;36], and GlcN6P (glucosamine-6-phosphate) [37].

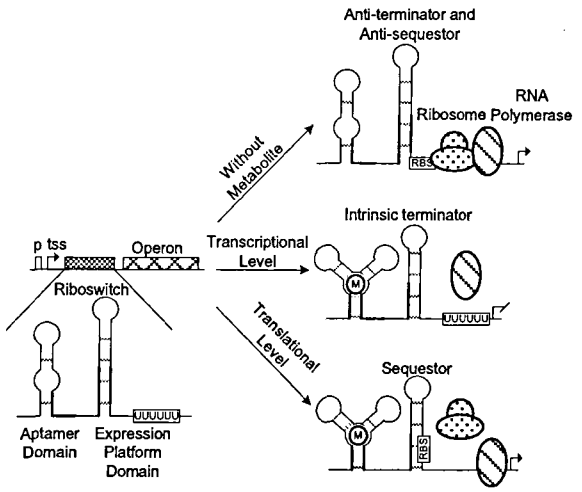

Figure 1: Simplified model of riboswitch. (*Left*) Structurally, riboswitch comprises aptamer and expression platform domains that allosterically regulate gene expression of its adjacent protein-coding operon. (*Right, top*) During insufficiency of target metabolite, the stem-loop folds into anti-terminator and anti-sequestor so that the ribosome follows the RNA polymerase to complete the read-through of protein-coding operon. When the target metabolite binds to the aptamer domain, gene regulation can occur at either the transcriptional or the translational level. (*Right, middle*) The stem-loop folds into an intrinsic terminator causing premature transcription. (*Right, bottom*) The stem-loop folds into a sequestor to prevent translation initiation by occluding the ribosome-binding site. *p, promoter; tss, transcription start site; RBS, ribosome-binding site; M, metabolite.*

In this paper, we offer a mathematical model of the *E. coli* TPP riboswitch's regulatory system, that has yet been published or reported, in order to investigate the nonlinear dynamic behavior of *E. coli* TPP riboswitches. The driving motivation arises from the recent discovery that homologs of the *E. coli* TPP riboswitch have been identified in fungal and plant mRNAs, where they bind to TPP with affinities comparable to those of their prokaryotic counterparts [28]. This further suggests that TPP riboswitches may also exist in mammalian cellular systems, which if proven valid may reinforce the hypothesis that "modern" riboswitches are reminiscence of ancient genetic regulatory systems from the "RNA world" [38].

This paper is organized as follows. Section 2 briefly describes the TPP biosynthesis pathway in *E. coli*, and TPP riboswitches in regulating TPP biosynthesis genes and operons. Section 3 develops a mathematical model of the TPP riboswitch's regulatory system so as to simulate the time-course experiments of TPP biosynthesis in *E. coli*. In section 4, the simulation results are validated against three sets of reported experimental data in order to gain insight into the nature of steady states and the stability of TPP riboswitches, and to explain the biological significance of regulating at level of transcription or translation, or even both. Section 5 concludes with a discussion on the feasibility of the proposed model, and possible future directions.

2 BACKGROUND OF TPP BIOSYNTHESIS PATHWAY

In Figure 2, fourteen *thi* genes [39] have been identified in the TPP biosynthesis pathway of *E. coli*, to participate in the biosynthesis, salvage, and transport of TPP. They are arranged into three operons of *thiCEFSGH* [40], *thiMD* [40;41], and *thiBPQ* [42] as well as four single gene loci of *thiL* [43], *thiK*, *thiI* [44], and *dxs* [39].

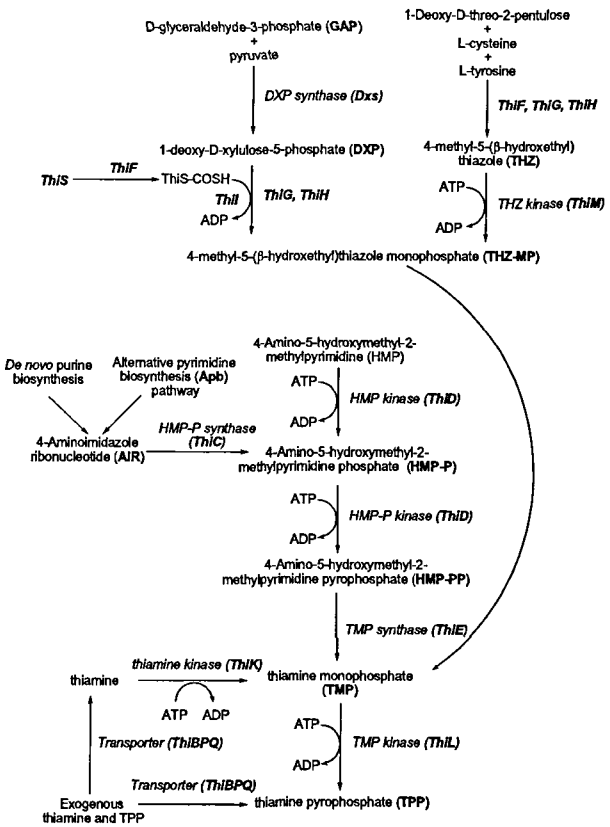

Figure 2: *E. coli* TPP biosynthetic pathway. Gene product names are *bold italics*. Abbreviated chemical names are in *parenthesis*.

Three kinases are involved in the salvage pathway of thiazole, pyrimidine, and thiamine from the culture medium. *ThiM* [41] phosphorylates the thiazole alcohol from THZ to THZ-MP, *ThiD* [45] catalyzes the phosphorylation of the pyrimidine alcohol from HMP to HMP-PP, and *ThiK* phosphorylates salvaged exogenous thiamine into TMP. *ThiC* [40] is involved in the pyrimidine biosynthesis of the HMP-P from AIR. Six genes products *Dxs, ThiI*, and *ThiFSGH* are required for the thiazole biosynthesis. *Dxs* [39] catalyzes the condensation of GAP and pyruvate to produce DXP. *ThiI* [44] catalyzes the transfer of the sulfur of cysteine to *ThiS*, while *ThiF* [46] catalyzes the adenylation by ATP of the carboxyl-terminal glycine of *ThiS* [46]. *ThiS* thiocarboxylate performs as the sulfur carrier in TPP biosynthesis. *ThiGH* [47-49] complex is involved in the last step of the thiazole biosynthesis - a cyclization reaction requiring sulfur transfer to DXP from either the *ThiS* thiocarboxylate or the *ThiFS* conjugate, condensation of tyrosine, and cyclization to the thiazole product. Finally, *ThiE* [46] links the pyrimidine HMP-PP and thiazole THZ-P together into TMP, which is

phosphorylated by *ThiL* [43] into TPP. *ThiBPQ* [42] is involved indirectly in the biosynthetic pathway, by playing an important role in the transport of exogenous thiamine and TPP.

Until recently, no TPP dependent regulatory proteins have been identified in *E. coli*, to participate in the control of the three operons. Interestingly, each operon possesses and is modulated by a highly conserved 39 nucleotides region at 5' terminus to the start of translation. This "thi-box" was known to interact *in some ways* with TPP, *without* involving any protein-cofactors [42]. For example, time-course experiments of thiamine uptake in *E. coli* [50] demonstrated that thiamine was actively transported by a thiamine-binding protein (encoded by *thiBPQ*), whose activity was *mysteriously* repressed by excess thiamine. In contrast, the four single gene loci do not possess the thi-box, and are not regulated by TPP [43;44] possibly due to their involvement in additional biosynthetic pathways [39].

Experimental evidence [25] proved the hypothesis [43] that TPP acts *solely* in down-regulating the expressions of *thiMD* (at the translational level) and of *thiCEFSGH* (at both levels of transcription and translation) operons, through direct interaction with their corresponding upstream thi-boxes, *without* involving protein cofactors. Thi-boxes of *thiMD* and *thiCEFSGH* bind TPP with an apparent dissociation constant of 600 ηM and 100 ηM, respectively, more readily than TMP or thiamine. Thiamine analogues with less similarity to TPP fail to induce structural modulation in the thi-box. These findings strongly indicate that the thi-box possessing remarkable affinity and specificity for TPP, is an aptamer domain of TPP riboswitch.

3 THE MODEL

3.1 Mathematical Formulation and Assumptions

We constrain the mathematical model to the last three steps of TPP biosynthesis pathway in *E. coli*. In Figure 3, two genes *ThiD* and *ThiE* are involved in the chemical reaction of HMP-P→HMP-PP→TMP. The input substrate HMP-P is phosphorylated by *ThiD* into HMP-PP, which is converted by *ThiE* into TMP. TMP is finally phosphorylated into the output product TPP by *ThiL* that is unregulated by TPP.

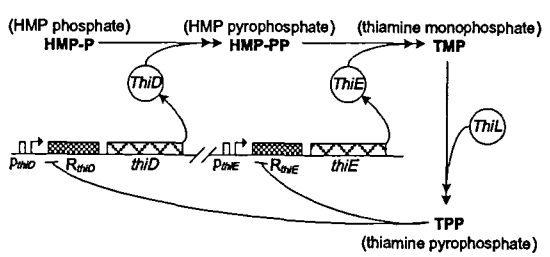

Figure 3: Abridged *E. coli* TPP biosynthetic pathway from HMP-P→HMP-PP→TMP→TPP. *p, promoter; R, riboswitch; HMP, 4-Amino-5-hydroxymethyl-2-methylpyrimidine.*

Our proposed model is based on two assumptions. (1) A relatively large population of identical molecules and cells at statistical equilibrium, because the derivation of the ODE-based model relies on the "global" average parameters. (2) The binding of the TPP metabolite to the aptamer domain of TPP riboswitch is assumed a rate-limiting step.

3.2 Model Equations

The rate of change in the concentration of the i^{th} transcribed gene $m_i(t)$ and its translated protein $p_i(t)$ can be generalized as,

$$\dot{m}_i(t) = \lambda_i c_i(t) - \alpha_i m_i(t) \quad (1)$$

$$\dot{p}_i(t) = \gamma_i d_i(t) - \beta_i p_i(t) \quad (2)$$

Where $i \in \{thiD, thiE\}$; both λ_i and γ_i represent the rates of transcription and translation of the i^{th} gene, respectively; $c_i(t)$ and $d_i(t)$ denote the fraction of DNA templates and the i^{th} transcribed gene, respectively, that are committed to the full transcription and translation of the i^{th} gene; α_i and β_i are the degradation rates of the i^{th} transcribed gene and its corresponding translated protein, respectively.

$$c_i(t) = \begin{cases} D, \text{ for } i = thiD \\ \dfrac{K_{TR,i} D}{K_{TR,i} + T(t)}, \text{ for } i = thiE \end{cases} \quad (3)$$

$$d_i(t) = \begin{cases} \dfrac{K_{TL,i} m_i(t)}{K_{TL,i} + T(t)}, \text{ for } i = thiD \\ m_i(t), \text{ for } i = thiE \end{cases} \quad (4)$$

Where D denotes the average copy of each DNA template; K_{TR} and K_{TL} represent the equilibrium dissociation constants associated with the regulation at the levels of transcription and translation, respectively. The rate of change in the concentration of the endogenous j^{th} metabolite $M_j(t)$, where $j \in \{HMP\text{-}PP, TMP, TPP\}$, is proportional to the expression level of $p_i(t)$, and the degradation-consumption rate of ε_j.

3.3 Parameter Values and Initial Conditions

From the mathematical model, the nonlinear differential equations are subjected to the parameter values summarized in Table 1, and initialized to the conditions given in equation (5).

Parameter	Value
Average volume of a cell	8.0×10^{-16} liters [11]
Average growth rate in a minimal medium	1.0×10^{-2} min^{-1} [11]
λ, rate of transcription per template†	6.23×10^{-2} ηM min^{-1}
ν, rate of translation†	10.38×10^{-2} ηM min^{-1}
D, average copy of each DNA template	1.6 [11]
K_{TR}, equilibrium dissociation constant	10.35 ηM [25]
K_{TL}, equilibrium dissociation constant	62.1 ηM [25]
α, average mRNA degradation rate‡	$\sim 4.62 \times 10^{-2}$ min^{-1}
β, average protein degradation rate‡	$\sim 3.85 \times 10^{-3}$ min^{-1}
j^{th} metabolite degradation-consumption rate	~0 min^{-1}

Table 1: Parameter values used in the simulation for each *E. coli* cell at 37 °C. We assume, initially, each gene contributes on the average three mRNA and five protein molecules to a cell. †, values of λ and ν depend on the average growth rate of 1.0×10^{-2} min^{-1} for both *thiD* and *thiE*. ‡, values of $\alpha = \ln 2/\rho$ and $\beta = \ln 2/\pi$ assume the average half-lives of mRNA and protein molecules are 15 min and 180 min, respectively.

$$m_i(0) = 6.23\ \eta\text{M},\ p_i(0) = 10.38\ \eta\text{M},\ i \in \{thiD, thiE\}$$
$$M_j(0) = 0, j \in \{HMP\text{-}PP, TMP, TPP\} \quad (5)$$

4 SIMULATION RESULTS AND DISCUSSION

Our proposed model is validated against two experimentally proven test-cases. The first test-case comprises two independent sets of time-course experimental data for *thiD* [51] and *thiE* [52] in *E. coli*, involved in the synthesis of HMP-PP and TMP, respectively. Figure 4 shows that there is a reasonable qualitative agreement between the two data sets and the time-course simulation results of synthesized HMP-PP and TMP concentrations, for duration of 30 min.

Given the simplifying assumptions inherent to the model, we prolong the simulation to 1200 min in order to validate the accuracy of the steady-state values. Figure 5 shows the time-course simulation results of mRNA concentrations for *thiD* and *thiE*, for duration of 1200 min. Both mRNA concentrations decrease rapidly during the first 100 and 200 min approximately, before reaching their steady-state values. Steady-state mRNA concentration of *thiE* is substantially lower than that of *thiD*, demonstrating that the activity of *thiE* is significantly down-regulated by the TPP riboswitch at the transcriptional level, in comparison to that of *thiD*. This finding also indicates that in *E. coli*, *thiD* is not significantly down-regulated at the transcriptional level by TPP riboswitch, but possibly and primarily at the translational level [25].

Figure 6 and Figure 7 depict the time-course simulation results of protein concentrations for *thiD* and *thiE*, as well as their synthesized HMP-PP and TMP concentrations, for duration of 1200 min. Both protein and metabolite concentrations increase rapidly during the first 100 and 200 min approximately, and then undergo maxima before declining gradually to their steady-state values. Overall concentrations of protein and output product HMP-PP of *thiD* are slightly higher (in value) and are relatively slower (in rate of decrease), than that of *thiE*. Both observations suggest that in *E. coli*, *thiD* and *thiE* are down-regulated at the translational level by TPP riboswitches, but with different physiological effects [25].

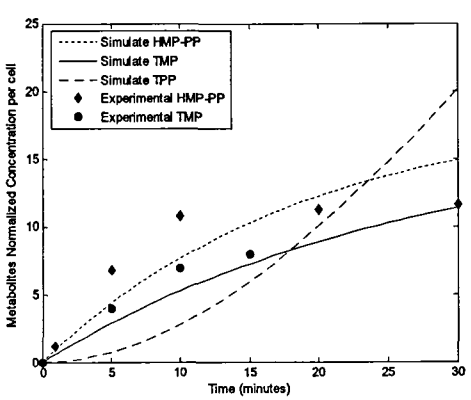

Figure 4: Comparison of the simulation results against the time-course experimental data, for duration of 30 min. ♦, the concentration of HMP-PP synthesized by *thiD* [51]; •, the concentration of TMP synthesized by *thiE* [52].

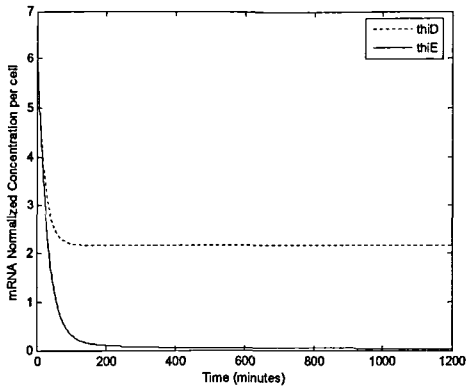

Figure 5: Time-course simulation results of mRNA concentrations for *thiD* and *thiE*, for duration of 1200 min.

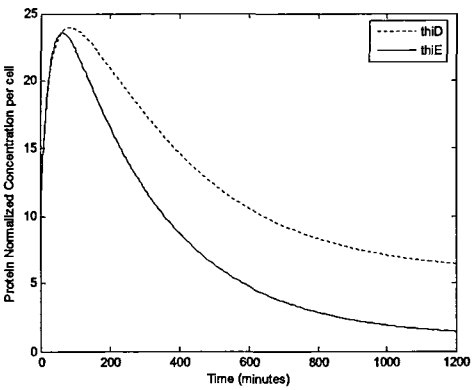

Figure 6: Time-course simulation results of protein concentrations for *thiD* and *thiE*, for duration of 1200 min.

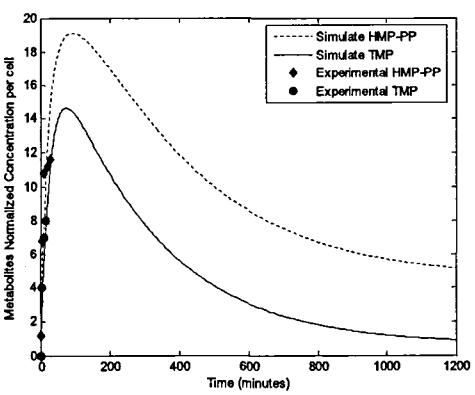

Figure 7: Time-course simulation results of concentrations for synthesized HMP-PP and TMP, for duration of 1200 min. ♦, the concentration of HMP-PP synthesized by *thiD* [51]; •, the concentration of TMP synthesized by *thiE* [52].

In the second test-case, the simulation results (see Figure 5 and Figure 6) are validated against experimental data [25] for *thiMD* and *thiCEFSGH* operons. As summarized in Table 2, there is a reasonable qualitative agreement between the experimental data and the simulation results at both levels of transcription and translation. TPP riboswitch down-regulates expression of *thiMD* mainly at the translational level and of *thiCEFSGH* at both levels [25]. Our findings also indicate

that in the TPP biosynthesis pathway of *E. coli*, the biological effect of down-regulating *thiE* at the translational level by TPP riboswitch is less prominent than that at the transcriptional level. Possibly in *E. coli*, the highly conserved TPP riboswitches have evolved to be more metabolically frugal and efficient, in order to cease prematurely transcription of a long and multi-gene TPP biosynthetic operon (e.g. *thiCEFSGH*) if its products are not needed. This relationship may also suggest that operons (e.g. *thiCEFSGH*) possessing higher number of biosynthetic genes are more likely to be regulated by a transcriptional termination system, while those operons (e.g. *thiMD*) with fewer genes are preferably regulated by a translational inhibition mechanism. Further investigations are needed to determine the correlation between the frequency of genes within an operon and the types of genetic regulatory mechanisms being used by the operon.

	Transcriptional Level		Translational Level	
Operon	*Simulation*	*Experiment*†	*Simulation*	*Experiment*‡
thiD	5.8/2.2 = 2.6	~0	24.0/6.0 = 4.0	18
thiE	5.8/0.2 = 29.0	16	23.5/1.5 = 15.7	110

Table 2: Comparison of the simulation results against the experimental data [25] at transcriptional and translational levels. (*Simulation columns*) Values of numerators and denominators are estimated maximum and steady state values, respectively, from Figure 5 and Figure 6. (*Experiment columns*) †, transcriptional fusion construct of *thiD* is not suppressed by thiamine, while that of *thiE* is down-regulated by 16-fold. ‡, *thiD* and *thiE* translational fusion constructs exhibit thiamine-dependent suppression of 18- and 110-fold, respectively.

5 CONCLUSION

The main contribution of this paper is, we have developed a nonlinear dynamic model for the *E. coli* TPP riboswitch's regulatory system possessed by *thiCEFSGH* and *thiMD* operons, so as to simulate the time-course experiments of TPP biosynthesis in *E. coli*. By validating the simulation results against available biological information pertaining to the processes of transcription and translation of both operons, we have derived a "first-order" approximation of TPP riboswitch's regulatory activity in terms of its basic biological function and its resulting biologically relevant responses.

Though we have adopted deterministic modeling for our preliminary study of *E. coli* TPP riboswitch, we have concerns whether this may be an appropriate long-term approach. As mentioned in [53], deterministic model assumes the concentration of the reacting species populates to a relatively high level and varies time-continuously along a fixed trajectory from its initial state without any abrupt discontinuities. Thus, deterministic model may be unable to capture "spike" phenomena caused by potential inherent randomness when riboswitch switches "on" or "off". Such model is also cumbersome for modeling relatively small population of a particular species as the stochastic effect amplifies with the decrease in the population, especially so when naturally found riboswitches may be fewer in numbers than protein factors. We are currently exploring "stochastic modeling" of a single-molecule riboswitch, which will consider uncertainties inherent to the processes of transcription and translation initiation, elongation, and pause-termination due to conformational change of stem-loop structures.

ACKNOWLEDGMENT

This work is supported by the Bioinformatics Institute (Singapore), and the Agency for Science, Technology and Research (Singapore).

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