Modeling

Kill Switch Model

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Kill Switch Model

Q&A

1. Can we control the amount of CI protein?

The amount of CI protein is controllable. We can change RBS strength to change mRNA translation rate or add degradation tag to change the protein degradation rate.

2. How to explain the effectiveness of the kill switch?

The engineered *Bacillus subtilis* did not commit suicide in the laboratory and in the intestine of earthworms because CI repressor protein can bind to the specific inhibition binding site of promoter P_{CI} to prevent production of switch RNA. The kill switch cannot be turned on. When they were expelled out of the intestine, CI protein and Trigger RNA were no longer produced and always degraded. As the amount of CI decreased, more and more free inhibition binding sites were exposed, more and more Switch RNA were produced, which can combine with Trigger RNA to produce MazF, making the engineered bacteria commit suicide.

3. Which is the best RBS and degradation tag combination of CI protein?

In the intestine of earthworms, the production of MazF is the key. Due to the possibility of leakage, the production of MazF is not always at zero. We simulated the leakage of MazF in the intestinal environment to find the combination that generated the least MazF If the combinations were not unique, considering the simplicity of the pathway design, we would choose the one adding the same degradation tag for CI produced both in the laboratory and in the intestine.

Content

1 Abstract

To avoid biological pollution, we modified the gene pathway of *Bacillus subtilis* by introducing kill switch making that the engineered bacteria do not commit suicide in the intestine, but switch on the suicide mode after it were expelled out of the intestine. To verify the feasibility of the kill switch, we built the Kill Switch Model via **MATLAB,** which combined the degradation rate of CI protein with different strength RBS. After we quantitatively simulated the effect of the kill switch under various combinations, a reasonable one was given. By adding **the LVA degradation tag** and selecting **B0029 RBS**, the kill switch can operate effectively.

2 Background

The level of MazF was affected by the RBS and the degradation rate of CI protein, so we chose to adjust the two to find the best combination.

- **Laboratory Culture Phase**

Add IPTG to induce the production of enough CI protein, no MazF.

- **In the Intestine of an Earthworm**

The accumulated CI protein induced in laboratory degraded, and in the absence of oxygen, *Bacillus subtilis* expressed CI protein and Trigger RNA, maybe MazF because of leakage.

- **External Environment**

When *Bacillus subtilis* was expelled out of the intestine. CI protein and Trigger RNA were no longer produced and always degraded. As the concentration of CI decreased and the number of free inhibition sites increased, more and more Switch RNA were produced, which can combine with Trigger RNA to produce MazF.

Here are three ideal stages involved in CI protein concentration and MazF.

Stage	Laboratory	In the Intestine	Out of the Intestine
CI	+	+	-
MazF	-	-	+

Note: + means existence; - means inexistence

3 Model Hypothesis

- The degradation rate of mRNA and protein in a certain amount is a constant.
- The mRNA is generated at a constant constitutive transcription rate.
- The copy number of plasmids is kept as a constant.
- Other species such as RNAP polymerases and ribosomes are kept constant as well.
- Reactions are at equilibrium, steady state, or quasi-steady state **错误!未找到引用源。**.
- Each RBS part has a native strength irrespective of the promoter and protein-coding part it can be used with, and the translation rate with the same RBS has a linear relationship with the mRNA length **错误!未找到引用源。**.
- Since the probability of gene mutation is very small, our model did not consider gene mutation.

4 Symbol Description

Symbol	Explanation	Value	Units
CI-1	CI protein induced in laboratory		
CI-2	CI protein produced in earthworm intestine		
$K_{mRNA-C1}$	The transcriptional rate of DNA_{CI}	**5.39**	min^{-1}
K_{TR}	The transcriptional rate of DNA_{TR}	**19.12**	min^{-1}
K_{TS}	The transcriptional rate of DNA_{TS}	**2.52**	min^{-1}

$\boldsymbol{K_{C1-2}}$	The translation rate of $\boldsymbol{mRNA_{CI}}$ in the intestine of earthworms	**4.84**	$\boldsymbol{min^{-1}}$
$\boldsymbol{K_{tr.ts}}$	The translation rate of generating MazF	**2.09**	$\boldsymbol{min^{-1}}$
$\boldsymbol{d_{mRNA-CI}}$	The degradation rate of $\boldsymbol{mRNA_{CI}}$	**0.1386**	$\boldsymbol{min^{-1}}$
$\boldsymbol{d_{TR}}$	The degradation rate of Trigger RNA	**0.0365**	$\boldsymbol{min^{-1}}$
$\boldsymbol{d_{TS}}$	The degradation rate of Switch RNA	**0.18**	$\boldsymbol{min^{-1}}$
$\boldsymbol{d_{C1-2}}$	The degradation rate of CI produced in earthworm intestine		
$\boldsymbol{d_{C1-1}}$	The degradation rate of CI induced in laboratory		
$\boldsymbol{G_{CI}}$	The copy number of CI protein	**500**	
$\boldsymbol{G_{TR}}$	The copy number of Trigger RNA	**500**	
$\boldsymbol{G_{TS}}$	The copy number of Switch RNA	**500**	
$\boldsymbol{V_{mRNA-\mathrm{CI}}}$	The formation rate of $\boldsymbol{mRNA_{CI}}$		
$\boldsymbol{V_{\mathrm{TR}}}$	The formation rate of Trigger RNA		
$\boldsymbol{V_{\mathrm{TS}}}$	The formation rate of Switch RNA		
$\boldsymbol{\varepsilon_1}$	The coefficient of CI protein and inhibitory site binding		
K_1	The binding constant of Trigger RNA and Switch RNA	$\boldsymbol{1 \times 10^5}$	
O_{CI-1}	The first inhibition site		
O_{CI-2}	The second inhibition site		
K_{CIo1}.	The equilibrium association constant between the repressor and O_{CI-1} inhibition sites	$\boldsymbol{1 \times 10^{11}}$[5]	$\boldsymbol{M^{-1}}$

$K_{CIo2\cdot}$	The equilibrium association constant between the repressor and O_{CI-2} inhibition sites	$\mathbf{1 \times 10^{9}}$[5]	$\boldsymbol{M^{-1}}$
$K_{CId\cdot}$	The equilibrium association constant between the repressor and other sites	$\mathbf{8.3 \times 10^{6}}$[5]	$\boldsymbol{M^{-1}}$
λ_{12}	The ratio of site 1 by DNA looping of repressor and site 2 complex	100	

Note: 1. Calculation method of transcription rate: The general transcription rate is 40~80 nt/s[2]. We took 80nt/s as the standard to calculate the transcription rate according to the length of DNA sequence.

2. Calculation method of translation rate: The general translation rate range is 10~20 aa/s[2]. Except for CI proteins, 20 aa/s was used as the standard to calculate the translation rate according to the mRNA sequence length. The CI protein translation rate at the highest RBS strength was set as 20 aa/s, and the translation rates at other RBS strengths were calculated in turn according to the intensity ratio.

5 Design

By regulating the amount of CI protein, we can control the kill switch. In the laboratory, we selected the concentration of IPTG which could produce the most CI protein. After entering the intestine, RBS strength and CI protein degradation rate were adjusted by using our model to achieve the minimum amount of MazF. In addition, the combination should meet the need that the engineered bacteria can commit suicide as soon as possible when they were expelled out of the intestine.

5.1 RBS and Degradation Tag

◆ RBS Selection

RBS sequence, also called SD (Shine-Dalgarno) sequence, is a key controlling the initiation of translation and the expression of proteins. Therefore, it determines the level of translation, increasing yield of target product [3]. We can change the production of CI protein by selecting suitable RBS strength.

To select a more suitable RBS, We selected RBS with different strengths, as shown in **Table 5.1.1**.

Table 5.1.1. Four types of RBS

Name of RBS	translation rate (min^{-1})

B0034	4.84
B0064	3.02
B0029	0.52
B0033	0.04

Note: Calculation method of translation rate: The general translation rate range is 10~20 aa/s[2]. The CI protein translation rate at the highest RBS strength was set as 20 aa/s, and the translation rates at other RBS strengths were calculated in turn according to the intensity ratio.

◆ CI Degradation Tags

The exact rate of protein degradation depends on a number of factors: the concentration of CLPXP and ClpAP protease and SspB medium; the stability of the protein; the km with the protease binds; the temperature, etc. But we can change the rate of degradation by adding tags. The tags can be recognized by the CLPX foldase and forms a complex with the ClpP protease. The last three residues of the tag determine the strength of the interaction with Clpx, thus determining the ultimate protein degradation rate.

We chose three tags: LVA, AAV and ASV. Under the LVA tag, the degradation rate of CI reached 0.1733 min^{-1} , and the degradation rate reached 0.0173 min^{-1} and 0.0087 min^{-1} due to AAV and ASV tag **错误!未找到引用源。** .

Bacillus subtilis expressed CI protein both in the laboratory and in the intestine of earthworms. We can control the degradation rate of both stages at the same time, and choose two degradation tags for the two stages. Respectively, we got some different degradation rate combinations, as shown in **Table 5.1.2**.

Table 5.1.2. C1 degradation rate combinations

CI-2 \ CI-1	LVA	AAV	ASV
LVA	0.1733	0.0173/ 0.1733	0.0087 /0.1733
AAV	0.1733/ 0.0173	0.0173	0.0087 / 0.0173
ASV	0.1733 / 0.0087	0.0173 / 0.0087	0.0087

5.2 CI Rules

Since CI proteins were produced to form dimer and inhibitory site binding, in this section we had formulated rules for such binding, with CI2 representing the dimer CI repressor and O_{CI-1} and O_{CI-2} representing the two CI inhibition sites **错误!未找到引用源。**.

Dimer CI repressor

$$CI2_f + O_{CI-1f} \rightleftharpoons \mathrm{CI2}: O_{CI-1}$$

$$CI2_f + O_{CI-2f} \rightleftharpoons \mathrm{CI2}: O_{CI-2}$$

$$\mathrm{CI2}: O_{CI-1} + O_{CI-2f} \rightleftharpoons O_{CI-1}: \mathrm{CI2}: O_{CI-2} \rightleftharpoons \mathrm{CI2}: O_{CI-2} + O_{CI-1f}$$

$$CI2_f + D \rightleftharpoons \mathrm{CI2}: D$$

Operating area 1

$$CI2_f + O_{CI-1f} \rightleftharpoons \mathrm{CI2}: O_{CI-1}$$

$$\mathrm{CI2}: O_{CI-1} + O_{CI-2f} \rightleftharpoons O_{CI-1}: \mathrm{C12}: O_{CI-2} \rightleftharpoons \mathrm{CI2}: O_{CI-2} + O_{CI-1f}$$

Operating area 2

$$CI2_f + O_{CI-2f} \rightleftharpoons \mathrm{CI2}: O_{CI-2}$$

$$\mathrm{CI2}: O_{CI-1} + O_{CI-2f} \rightleftharpoons O_{CI-1}: \mathrm{CI2}: O_{CI-2} \rightleftharpoons \mathrm{CI2}: O_{CI-2} + O_{CI-1f}$$

Relationship:

$$[\mathrm{CI2}] = [CI2_f] + [\mathrm{CI2}: O_{CI-1}] + [\mathrm{CI2}: O_{CI-2}] + [O_{CI-1}: \mathrm{CI2}: O_{CI-2}] + [\mathrm{CI2}: D]$$

$$[O_{CI-1}] = [O_{CI-1f}] + [\mathrm{CI2}: O_{CI-1}] + [O_{CI-1}: \mathrm{CI2}: O_{CI-2}]$$

$$[O_{CI-2}] = [O_{CI-2f}] + [\mathrm{CI2}: O_{CI-2}] + [O_{CI-1}: \mathrm{CI2}: O_{CI-2}]$$

Where:

$$[\mathrm{CI2}: O_{CI-1}] = K_{CIo1}.[CI2_f].[O_{CI-1f}]$$

$$[\mathrm{CI2}: O_{CI-2}] = K_{CIo2}.[CI2_f].[O_{CI-2f}]$$

$$[O_{CI-1}: \mathrm{CI2}: O_{CI-2}] = K_{CIo2}.\lambda_{12}[\mathrm{CI2}: O_{CI-1}].[O_{CI-2f}] = K_{CIo1}.[\mathrm{CI2}: O_{CI-2}].[O_{CI-1f}]$$

$$[\mathrm{CI2}:D] = K_{CId}.[CI2_f].[D_f]$$

$$[\mathrm{D}] = [\mathrm{CI2}:D] + [D_f]$$

Note： CI2 represents the dimer CI repressor; O_{CI-1} and O_{CI-2} represent the two CI inhibition site; A_f represents A is free; A:B means A binds to B inhibition site; B:A:C means B and C inhibition sites are both occupied; K_{CIo}.. is the equilibrium association constant between the repressor and sites; λ_{12} means the ratio of site 1 by DNA looping of repressor and site 2 complex; D means other binding sites; [·] means concentration.

6 Modeling

6.1 In the Intestine

Constitutive expression, not affected by time, place, or environment, has no spatiotemporal specificity. The gene that encodes the protein is not dependent on any transcription factor, so it will continuously transcribe mRNA molecules, and then the translation of the protein will be out of control **错误!未找到引用源。**. The expression of CI protein in the intestine of earthworm can be easily described in **Figure 6.1**. The process of generating Switch RNA and Trigger RNA was similar to the process of producing $mRNA_{CI}$ in the figure.

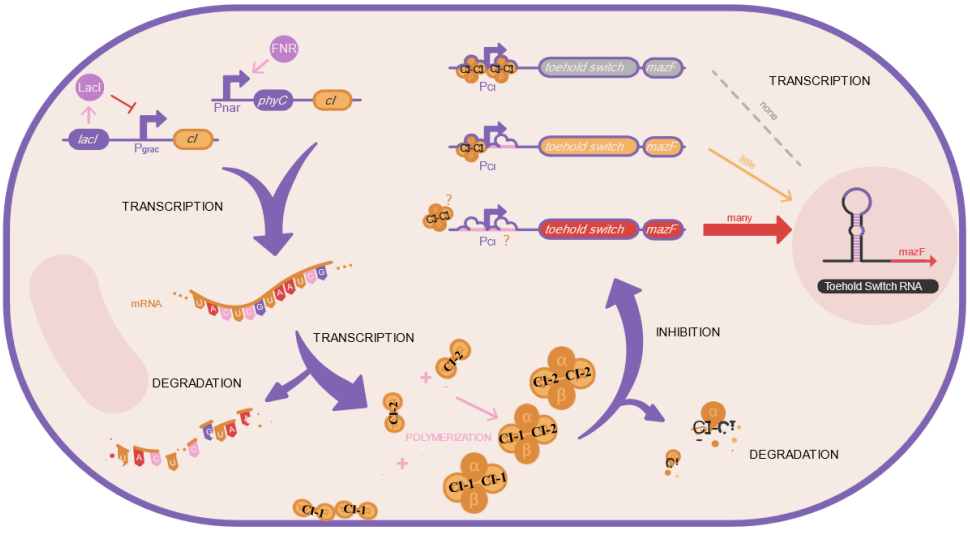

Fig.6.1. Production and Inhibition Effect of CI Protein

The CI protein, Switch RNA and Trigger RNA expression can be simply translated into the following biochemical reactions,

$$DNA_{C1} \xrightarrow{K_{mRNA-C1}} mRNA_{C1}$$

$$mRNA_{C1} \xrightarrow{K_{C1}} C1$$

$$mRNA_{C1} \xrightarrow{K_{mRNA-C1}} \emptyset$$

$$C1 \xrightarrow{d_{C1}} \emptyset$$

$$DNA_{TR} \xrightarrow{K_{TR}} Trigger\ RNA$$

$$Trigger\ RNA \xrightarrow{d_{TR}} \emptyset$$

$$DNA_{TS} \xrightarrow{K_{TS}} Switch\ \ RNA$$

$$Switch\ RNA \xrightarrow{d_{TS}} \emptyset$$

◆ 6.1.1 CI Protein Production

Here, we use **ordinary differential equation** to calculate the concentration of each substance. The concentration of $mRNA_{CI}$ can be expressed as follows:

$$\frac{d[mRNA_{C1}]}{dt} = V_{mRNA-C1} - [mRNA_{C1}]d_{mRNA-C1}$$

Where, $\boldsymbol{d_{mRNA-C1}}$ $(0.1386\boldsymbol{min^{-1}})$ is the degradation rate of $mRNA_{C1}$ and $V_{mRNA-C1}$ is the generation rate of $mRNA_{C1}$, which can be expressed as follows:

$$V_{mRNA-CI} = K_{mRNA-CI}\,[G_{CI}]$$

Where, $K_{mRNA-CI}$ is the transcription rate, and $K_{mRNA-C1} = 5.39\,min^{-1}$. $[G_{C1}]$ is the copy number of plasmids.

Here's what follows:

$$\frac{d[C1]}{dt} = V_{C1} - [CI-1]d_{Ci-1} - [CI-2]d_{CI-2}$$

Here, d_{CI-1} is the laboratory-induced degradation rate of CI protein, d_{CI-2} is the degradation rate of CI protein produced in earthworm intestines, both of which can be adjusted, and V_{C1} is the

production rate:

$$V_{C1} = K_{CI-2}\,[mRNA_{CI-2}]$$

$K_{CI-2} = 4.84\ min^{-1}$is the translation rate of CI generated in the intestine, which could be changed by adjusting RBS. The total amount of CI in intestine is shown in **Figure 6.1.1**.

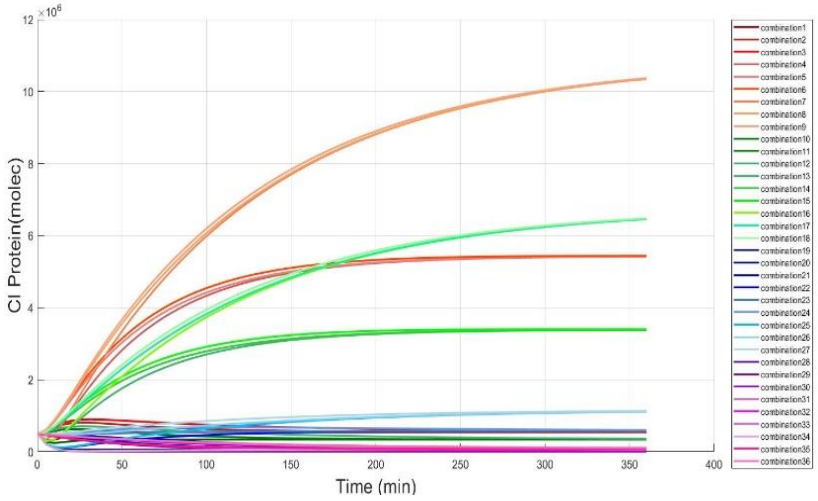

Fig.6.1.1. The Total Amount of CI in Intestine

◆ 6.1.2 Trigger RNA Production

Similarly, the concentration of Trigger RNA in the intestine can be calculated:

$$\frac{d[TR]}{dt} = V_{TR} - [TR]d_{TR}$$

Where $\boldsymbol{d_{TR}}$ is constant, $d_{TR} = 0.0365min^{-1}$ and V_{TR} is generation rate of $mRNA_{TR}$, given by the following formula:

$$V_{TR} = K_{TR}\,[G_{TR}]$$

$K_{TR} = 19.12\ min^{-1}$, $[G_{TR}] = 500.$ is the copy number of plasmids.

The amount of Trigger RNA in the intestine is shown in **Figure 6.1.2**.

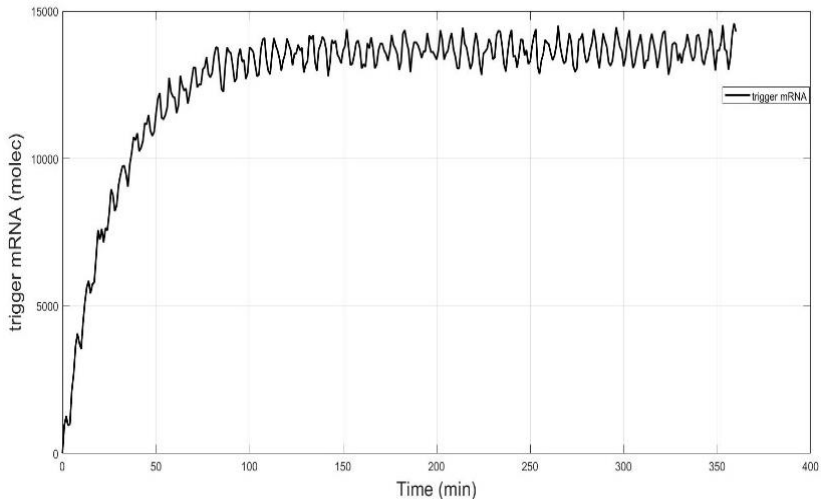

Fig.6.1.2. The Amount of Trigger RNA in the Intestine

◆ 6.1.3 Switch RNA Production

In order to solve the concentration problem of Switch RNA, we also assumed:

$$\frac{d[TS]}{dt} = V_{TS} - [TS]d_{TS}$$

Where d_{TS} is constant, $d_{TS} = 0.18min^{-1}$ and V_{TS} is generation rate of Switch RNA, given by the following formula:

$$V_{TS} = K_{TS}\,\varepsilon_1[G_{TS}]$$

$K_{TS} = 2.52\ min^{-1}$,$[G_{TS}] = 500$. ε_1 indicates the binding of CI and inhibition sites, which can be expressed as follows:

$$\boldsymbol{\varepsilon_1} = \left(\frac{[\boldsymbol{O_{C1-1f}}]}{[\boldsymbol{O_{C1-1}}]}\right)\left(\frac{[\boldsymbol{O_{C1-2f}}]}{[\boldsymbol{O_{C1-2}}]}\right)$$

The amount of Switch RNA in the intestine is shown in **Figure 6.1.3**.

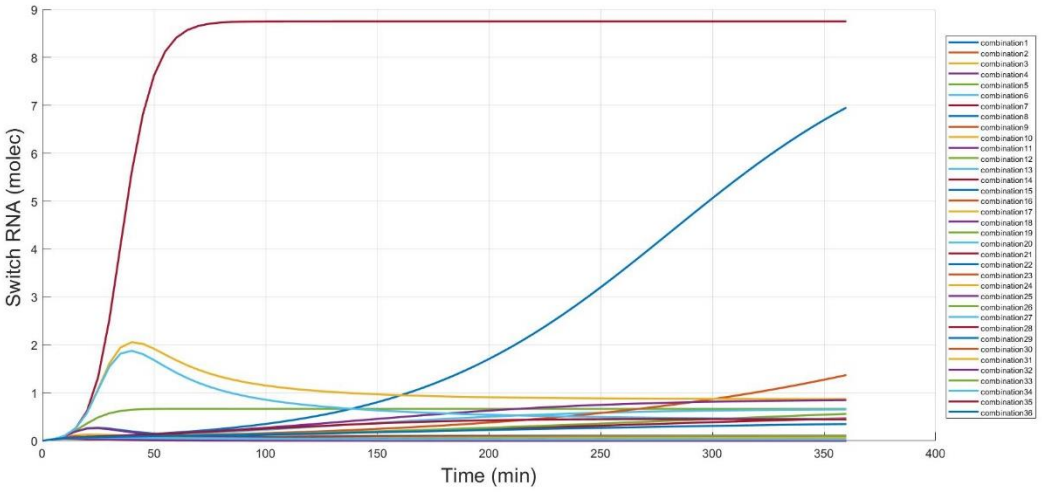

Fig.6.1.3. The Amount of Switch RNA in the Intestine

◆ 6.1.4 MazF Production

The expression of MazF in the intestine of earthworm can be easily described in **Figure 6.1.4.1**.

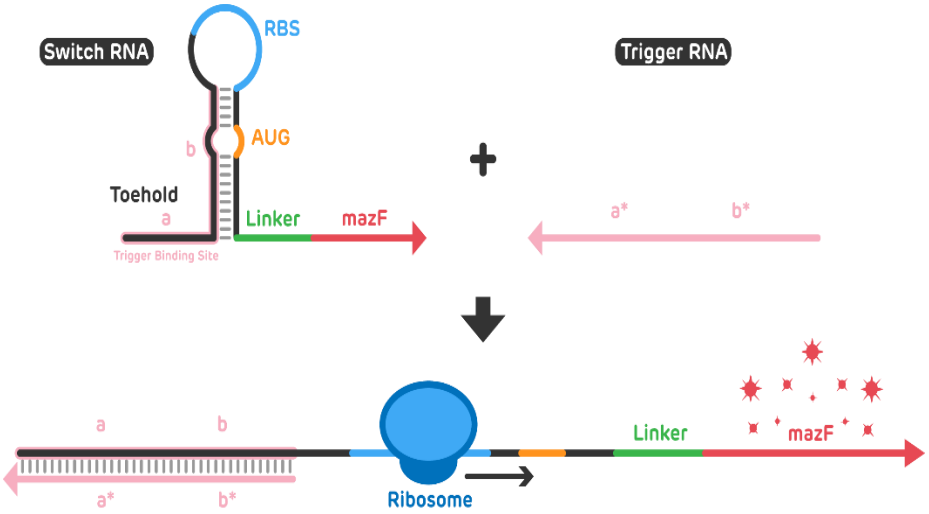

Fig.6.1.4.1. The Production of MazF

The specific process of generating MazF is represented by the following formula,

$$[trRNA] + [tsRNA] \rightleftharpoons [tr.tsRNA]$$

$$[tr.tsRNA] \xrightarrow{K_{tr.ts}} [mazF]$$

Among them, $K_{tr.ts} = 2.09\, min^{-1}$is the translation rate of generating MazF. $K_1 = 1 \times 10^5$ is the reaction equilibrium constant described as follows:

$$K_1 = \frac{[tr.tsRNA]}{[trRNA] \cdot [tsRNA]}$$

The total amount of Trigger RNA and Switch RNA are shown as follows,

$$[T_{tr}] = [trRNA] + [tr.tsRNA]$$

$$[T_{ts}] = [tsRNA] + [tr.tsRNA]$$

The amount of MazF in the intestine is shown in **Figure 6.1.4.2.**

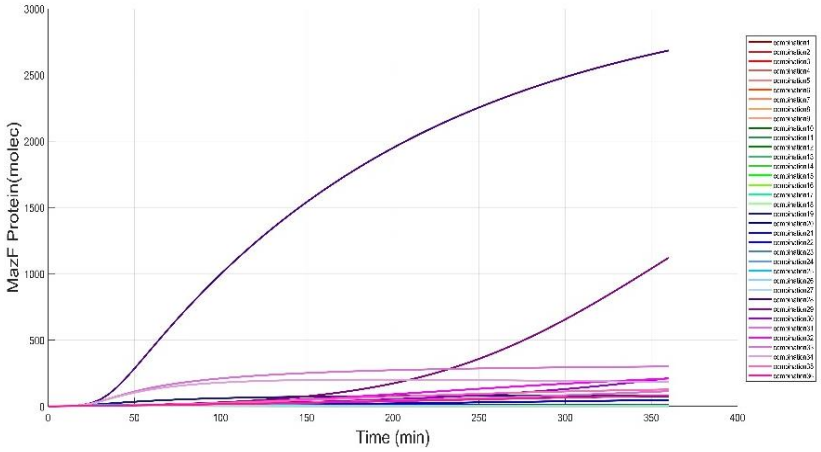

Fig.6.1.4.2. The Amount of MazF in the Intestine

6.2 Out of the Intestine

After the engineered bacteria were expelled out of the intestine, Trigger RNA and CI protein were no longer produced and always degraded. Switch RNA was produced in the same way as in the intestine. And with the gradual degradation of CI, Trigger RNA and Switch RNA would combine to produce MazF. The expression of CI protein, Trigger RNA , Switch RNA and MazF were obtained by applying differential equations above, as shown below.

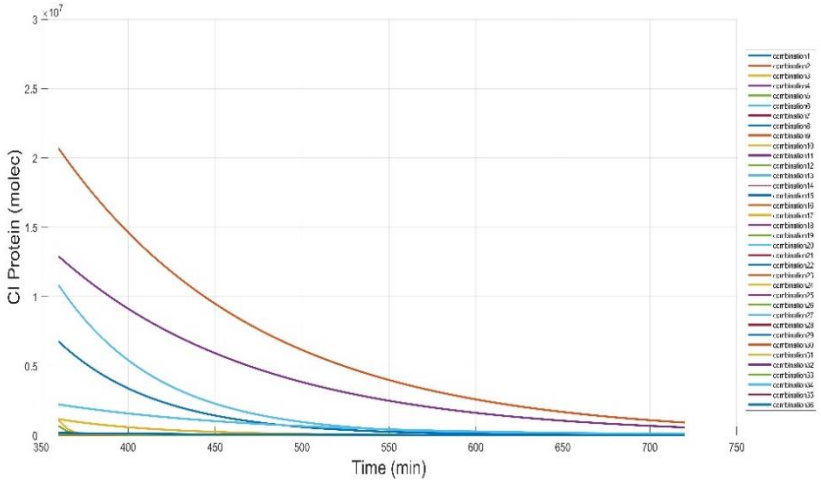

Fig. 6.2.1. The Total Amount of CI out of the Intestine

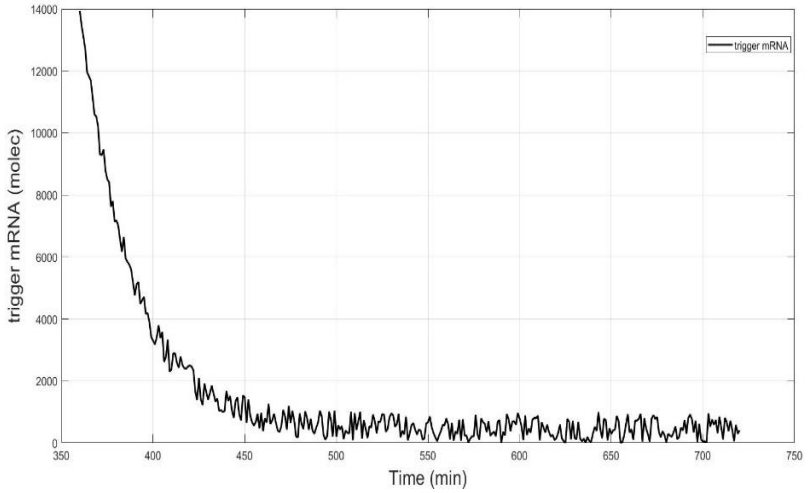

Fig.6.2.2. The Amount of Trigger RNA out of the Intestine

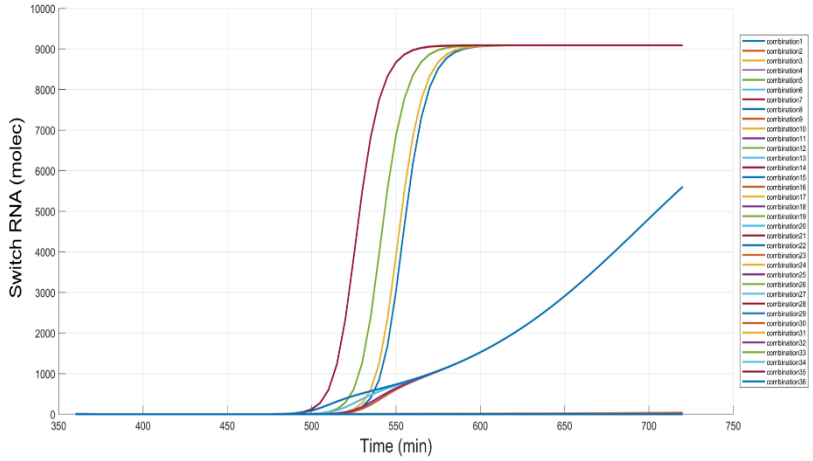

Fig.6.2.3. The Amount of Switch RNA out of the Intestine

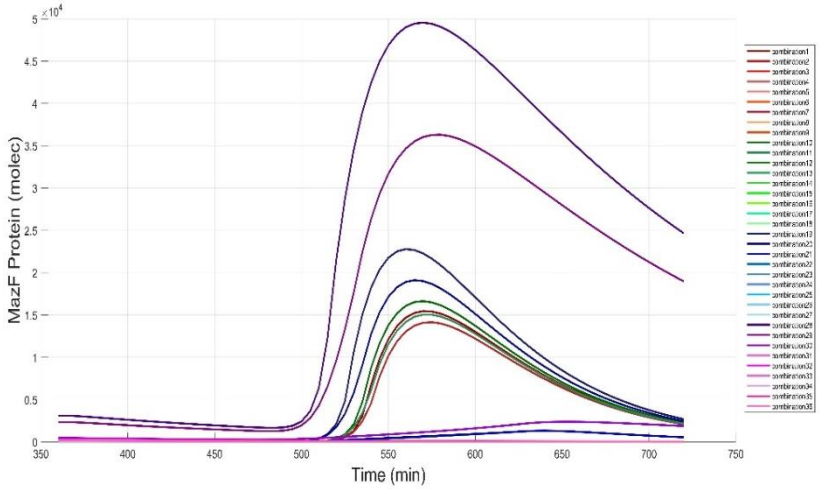

Fig.6.2.4. The Amount of MazF out of the Intestine

7 Results & Analysis

MazF directly determined whether the engineered bacteria will commit suicide or not. In order to ensure the kill switch to work, we analyzed the concentration of MazF under different combinations in and out of the intestine **Figure 7.1**.

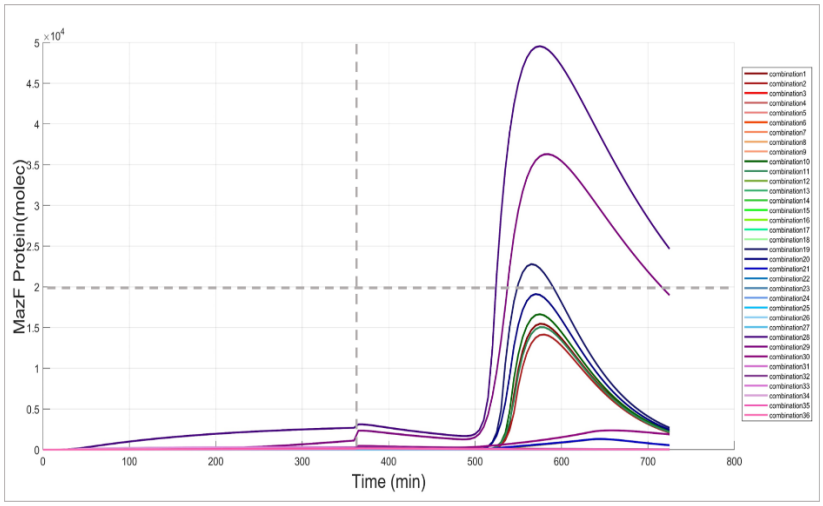

Fig.7.1. MazF Under Different Combinations in and out of the Intestine

We found that under the threshold set by us, only three combinations can meet the requirement of MazF generated in earthworm intestines less than the threshold of suicide, and far less than MazF generated out of the intestine. In addition, MazF generated out of the intestine is higher than the

threshold, which can make the engineered bacteria commit suicide.

Considering the simplicity of the pathway design, we hope to add the same degradation tag for CI-1 and CI-2. Therefore, our choice was: **B0029 RBS and LVA degradation tag.**

Under this combination, the quantities of various substances are shown in **Figure 7.2**.

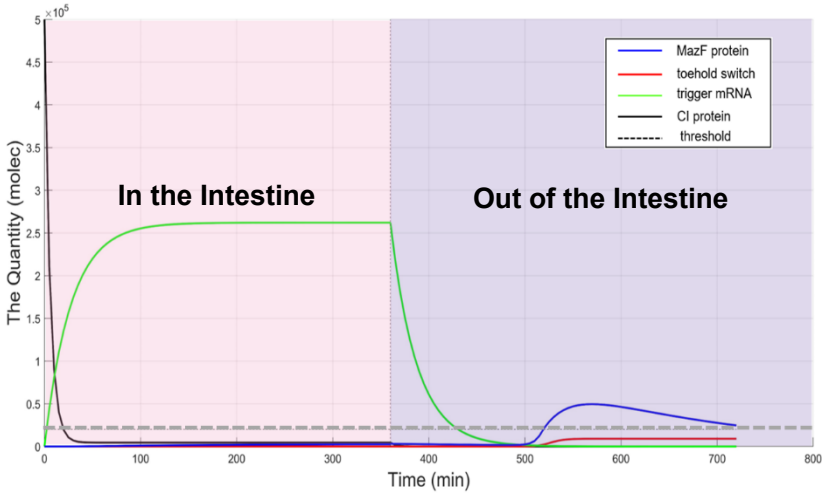

Fig.7.2. Various Substances Concentration

8 Sensitivity Analysis

We had known that the promoter strength can affect the transcription rate. In fact, data related to promoter strength is difficult to obtain. Therefore, sensitivity analysis was performed on promoter strength, making the transcription rate range from 2 min^{-1} to 6 min^{-1}, as shown in **Figure 8.1.**

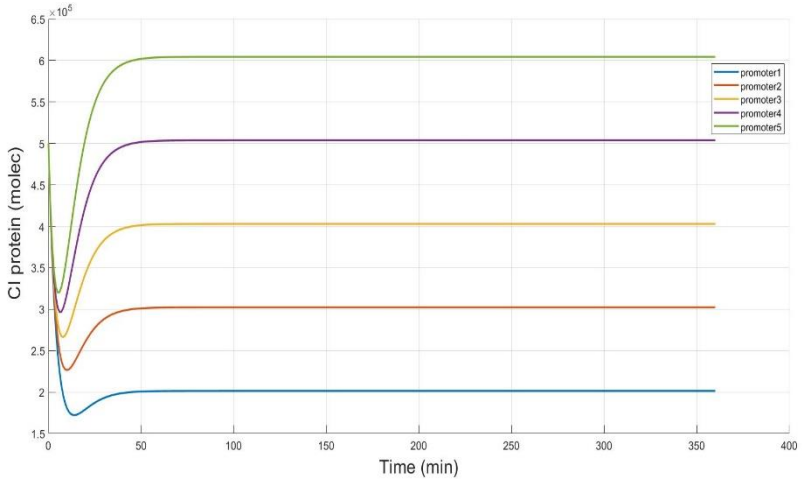

Fig.8.1. Sensitivity Analysis of Promoter Strength

The engineered bacteria were induced to produce CI proteins by IPTG in the laboratory. we had

selected the concentration of IPTG that could produce the most CI proteins to inhibit the production of Switch RNA. And in order to explore the influence of CI-1 concentration on the subsequent generation of CI-2 concentration, we conducted a sensitivity analysis on the CI protein concentration of engineered bacteria when they entered the earthworm intestine, as shown in **Figure 8.2.**

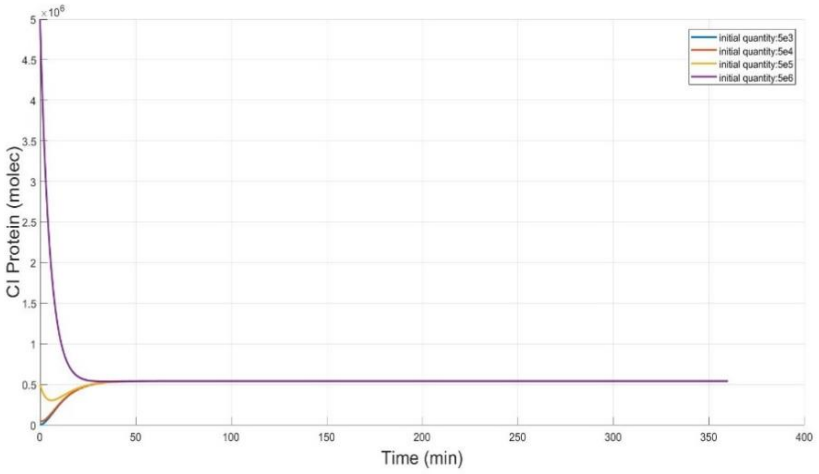

Fig.8.2. Sensitivity Analysis of CI Initial Concentration.

The results showed that:

1、The CI-2 protein production was sensitive with the promoter strength. But since the promoter strength is easy to change, we could only choose the best RBS strength.

2、The CI-2 protein production wasn't sensitive with quantity of CI induced in lab. Thus, our choice on the quantity of CI induced in lab is reasonable.

References

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