Labbook of dynamic CORN

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Abstract

This Labbook describes the development of metabolic dynamic models of CORN

1 Introduction

Modeling is a powerful tool in synthetic biology. It can provide us with an important engineering approach to characterize our pathways quantitatively and predict their performance, thus help us test and modify our design.

Through the dynamic model, we hope to gain insights of the characteristics of our whole circuit's dynamics. Several tools including ODEs and interpolation are employed.

2 Method

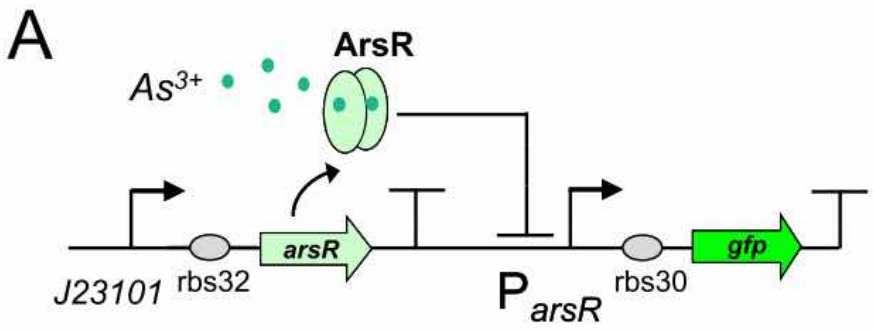

Figure 1: Schematic diagram of plasmid1

At the beginning, on the plasmid#1, the promoter P_{arsR} isn't bound with ArsR, thus it is active. $ArsR$ and $smURFP$ are transcribed and translated under the control of the promoters P_{arsR_u} and P_{arsR_d}, with subscript u and d representing upstream and downstream separately. The subscript l of smURFP in the equation

means leaky expression without the expression of As^{3+}. As ArsR is expressed gradually, it will bind with the promoter P_{arsR} and make it inactive.

$$P_{J23104} \xrightarrow{k_1} P_{J23104} + ArsR \tag{1}$$

$$P_{arsR_d} \xrightarrow{k_2} P_{arsR_d} + smURFP_l \tag{2}$$

$$ArsR + P_{arsR} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} ArsR * P_{arsR} \tag{3}$$

On the plasmid#2, the fusion protein of dCas9 and RNAP(RNA polymerase) are produced after transcription and translation, and $sgRNA$ is produced after transcription.

$$P_{tet} \xrightarrow{k_4} P_{tet} + dCas9 * RNAP \tag{4}$$

$$P_{tet} \xrightarrow{k_5} P_{tet} + sgRNA \tag{5}$$

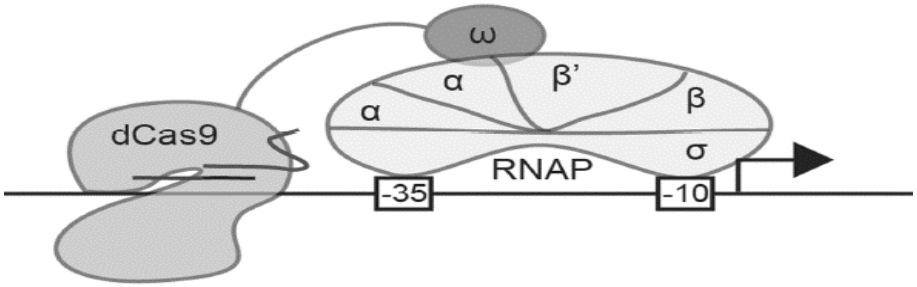

Figure 2: Schematic diagram of dCas9/RNAP

dCas9(*RNAP) can bind with its target DNA sequence without cutting, which is at the upstream of the promoter P_{arsR_d}. Simultaneously, dCas9 can lead RNAP to bind with the promoter P_{arsR_d} and enhance the transcription of smURFP. However, because the promoter P_{arsR_d} has already bound with ArsR, as a result, RNAP can't bind with the promoter P_{arsR_d} .

However, at the presence of As^{3+}, it can bind with ArsR, then dissociate ArsR and P_{arsR_d}, which makes the combination of RNAP and P_{arsR_d} possible.

(Declaration: $[dCas9/RNAP] = [dCas9] = [RNAP]$;
$[P_{arsR_d}] = [P_{arsR_u}] = \frac{1}{2}[P_{arsR}]$)

$$ArsR + As^{3+} \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} As^{3+} * ArsR \tag{6}$$

$$ArsR * P_{arsR} + As^{3+} \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} P_{arsR} + As^{3+} * ArsR \tag{7}$$

$$dCas9 * RNAP + sgRNA \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} dCas9 * RNAP : sgRNA \tag{8}$$

$$dCas9 * RNAP : sgRNA + P_{arsR_d} \underset{k_{-9}}{\overset{k_9}{\rightleftharpoons}} dCas9 * RNAP : sgRNA * P_{arsR_d} \tag{9}$$

$$dCas9 * RNAP : sgRNA * P_{arsR_d} \xrightarrow{k_{10}} dCas9 * RNAP : sgRNA * P_{arsR_d} + smURFP \tag{10}$$

We then take degradation into account:

$$ArsR \xrightarrow{k_{d1}} \emptyset \tag{11}$$

$$smURFP \xrightarrow{k_{d2}} \emptyset \tag{12}$$

$$ArsR * P_{arsR} \xrightarrow{k_{d3}} P_{arsR} \tag{13}$$

$$As^{3+} * ArsR \xrightarrow{k_{d4}} As^{3+} \tag{14}$$

$$dCas9 * RNAP \xrightarrow{k_{d5}} \emptyset \tag{15}$$

$$sgRNA \xrightarrow{k_{d6}} \emptyset \tag{16}$$

$$dCas9 * RNAP : sgRNA \xrightarrow{k_{d7}} \emptyset \tag{17}$$

$$dCas9 * RNAP : sgRNA * P_{arsR} \xrightarrow{k_{d8}} P_{arsR} \tag{18}$$

2.1 simulation

SimBiology toolbox provides functions for modeling, simulating, and analyzing biochemical pathways on basis of the powerful computing engine of Matlab.

COPASI is freeware developed withcollaboration of VBI and EMLR. It provides almost the same functions as SimBiology, though not quite powerful. But compared with SimBiology, it provides a friendly user interface for model analysis, such as parameter estimation,and parameter scan.

Through the figure, we can see that the smURFP fluorescence gradually increased and then reached a steady state after a period of time in the presence of arsenic ions.

Table 1: Parameters

Rate constants	Value	units
k1	1.999e-5	1/s
k2	3.312e-6	1/s
k3	3.3e7	1/M
k4	1.995e-5	1/s
k5	3.312e-6	1/s
k6	1.66e7	1/M
k7	1.26e4	1/s
k8	1.6e-2	1/s
k9	1.66e-5	1/s
k10	4e-5	1/s
kd1	3.07e-3	1/s
kd2	1e-5	1/s
kd3	1e-3	1/s
kd4	1.53e-3	1/s
kd5	2e-2	1/s
kd6	7.62e-3	1/s
kd7	1e-2	1/s
kd8	1e-1	1/s

3 References

1.Berset, Y. et al. Mechanistic Modeling of Genetic Circuits for ArsR Arsenic Regulation. ACS Synthetic Biology 6, 862–874 (2017).

2.Bikard, D.et al. Programmable repression and activation of bacterial gene expression using an engineered CRISPR-Cas system. Nucleic Acids Research 41, 7429–7437 (2013).

3.Cai, Y. et al. Modeling the arsenic biosensor system. BMC Systems Biology 1, P83 (2007).

4.Pola-López, L. A. et al. Novel arsenic biosensor "POLA" obtained by a genetically modified E. coli bioreporter cell. Sensors and Actuators B: Chemical 254, 1061–1068 (2018).

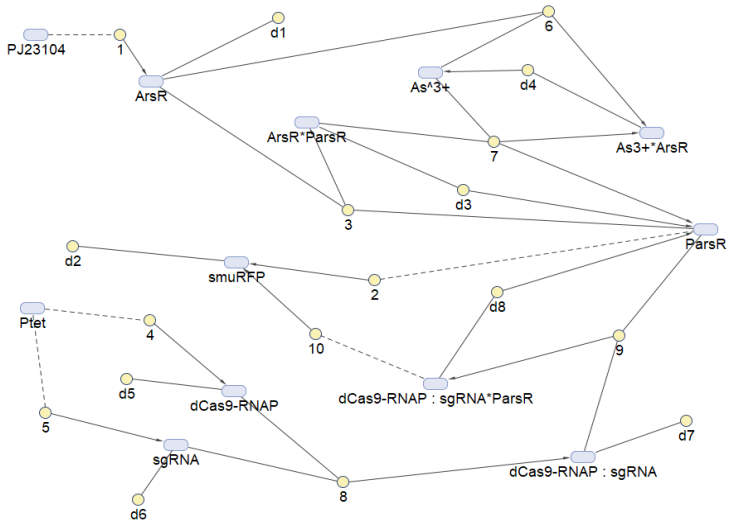

Figure 3: reaction map generated from the reaction set above using SimBiology Toolbox

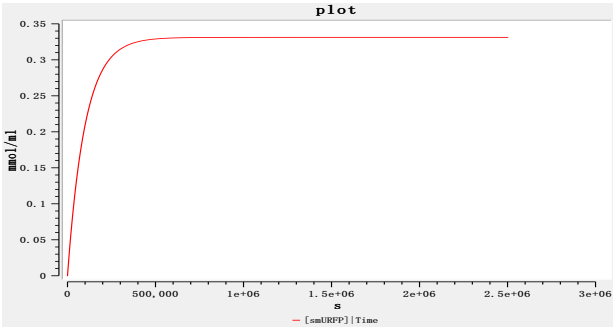

Figure 4: Schematic diagram of smURFP fluorescence by COPASI