Labbook of dynamic CORN

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Abstract

This Labbook describes the development of metabolic dynamic models of CORN

1 Brief introduction

Modeling is a powerful tool in synthetic biology and engineering. Mmodeling has provided us with an important engineering approach to characterize our pathways and predict their performance, thus helped us with modifying and testing our designing.

Through our model, we hope to gain insight of the gene expression dynamics of our whole circuit. And also we tried to better characterize our parts, analyze our experimental data,and protein transport and concentration changes throughout the whole process. Several tools including ODEs and interpolation are employed.

2 Kinetic model

2.1 analysis of the problem

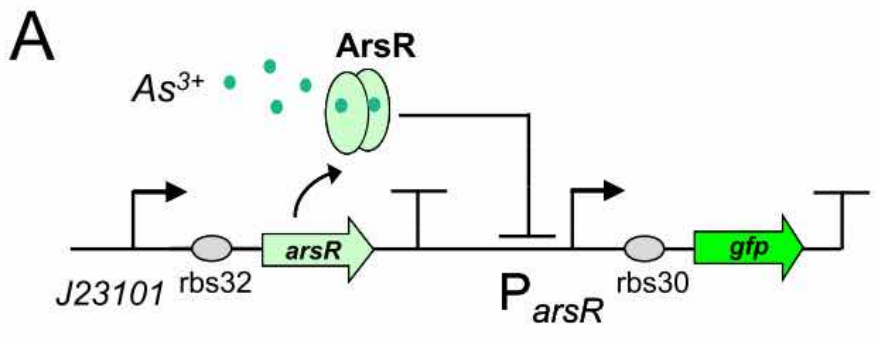

Figure 1: Schematic diagram of plasmid1

At the beginning, on the plasmid1, the promoter P_{arsR} isn't bound with ArsR, and thus active. And ArsR and $smURFP_l$ are transcribed from the promoter

P_{arsR}. $smURFP_l$ means leaking expression without the expression of As^{3+}.Then ArsR will bind with the promoter P_{arsR} and make it inactive. [**pola2018novel**]

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

$$P_{arsR} \xrightarrow{k_2} P_{arsR} + smURFP \tag{2}$$

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

On the plasmid 2, fusion protein of dcas9(dead Cas9, a mutant of Cas9) and RNAP(RNA polymerase) are produced after transcription.

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

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

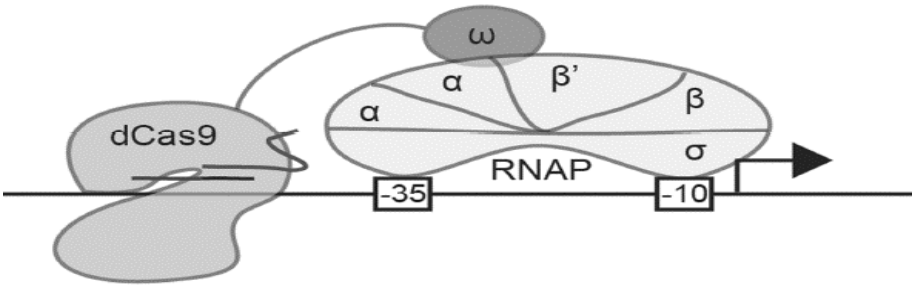

Figure 2: Schematic diagram of dCas9/RNAP

dCas9/RNAP can bind with it's target DNA sequence(upstream of promoter sequence) but not cut it. Simultaneously, dCas9 should have led RNAP to bind to the promoter P_{arsR_d} and enhanced the transcription of GFP. However, the promoter P_{arsR} has already bound with ArsR, as a result, RNAP can't bind with the promoter P_{arsR} .[**bikard2013programmable**]

However, at the presence of As^{3+}, As^{3+} can bind with ArsR, then dissociate ArsR and P_{arsR_d}, and combine RNAP and P_{arsR_d}.

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

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

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

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

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

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

Take the degration into account

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

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

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

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

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

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

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

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

2.2 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. Enter the above equations into copasi, set the initial concentration, parameter values etc., and you will get the result.

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	source
k1	1.999e-5	1/s	Berset et al.
k2	3.312e-6	1/s	Berset et al.
k3	3.3e7	1/M	Berset et al.
k4	1.995e-5	1/s	Estimated to be the same as in comparison to k1
k5	3.312e-6	1/s	Estimated to be the same as in comparison to k2
k6	1.66e7	1/M	Berset et al.
k7	1.26e4	1/s	Berset et al.
k8	1.6e-2	1/s	2017igem Munich
k9	1.66e-5	1/s	2017igem Munich
k10	4e-5	1/s	Estimated to be slow in comparison to k2
kd1	3.07e-3	1/s	Berset et al.
kd2	1e-5	1/s	Berset et al.
kd3	1e-3	1/s	Berset et al.
kd4	1.53e-3	1/s	Berset et al.
kd5	2e-2	1/s	Estimated to be fast in comparison to kd1
kd6	7.62e-3	1/s	Estimated according to Berset et al.
kd7	1e-2	1/s	Estimated to be slow in comparison to kd5
kd8	1e-1	1/s	Estimated to be slow in comparison to kd7

References

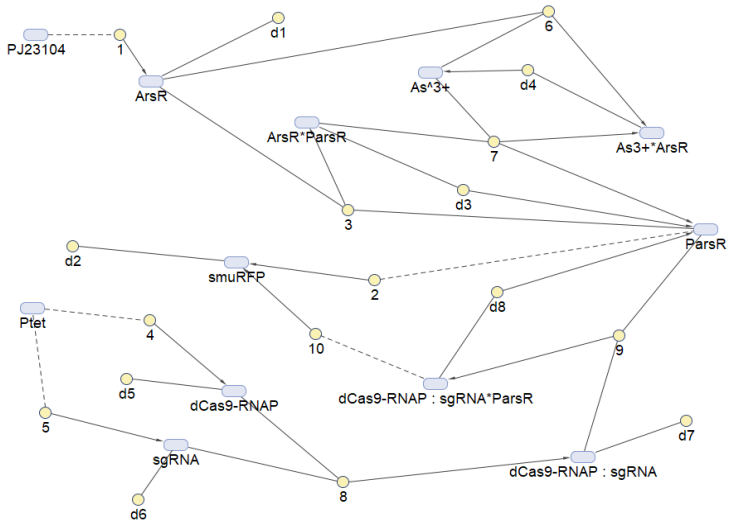

Figure 3: reaction map generated from the reaction set above using SimBiology Toolbox

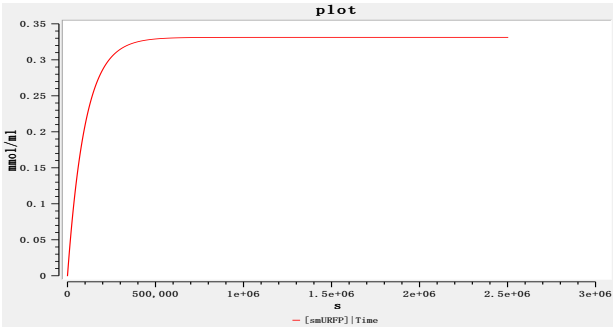

Figure 4: Schematic diagram of smURFP fluorescence by COPASI