

Labbook of dynamic CORN

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Abstract

This Labbook describes the development of metabolic dynamic models of CORN

1 Overview

Modeling is a powerful tool in synthetic biology and engineering. Modeling 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.

Basically, the models built by us can be divided into two parts.

On kinetic model parts, 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 Brief introduction

Concerned about the pollutions caused by heavy metals released by manufacturers and industries to the environment including air, water and soil and causing great harm to people's health, we are planning to utilise an engineered microorganism to detect the existence of heavy metals, aiming to develop a novel, highly sensitive and user-friendly detection device. We will engineer and construct detection and reporter circuits into *E. coli* and utilise CRISPR system to modify the expression of the reporter, smURFP, a kind of fluorescent protein. The change of the level of fluorescence released from smURFP will signal the existence of heavy metals. Our goal is to improve the sensitivity of the device to a great extent to guarantee the practicability and convenience of it.

3 Kinetic model

3.1 analysis of the problem

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

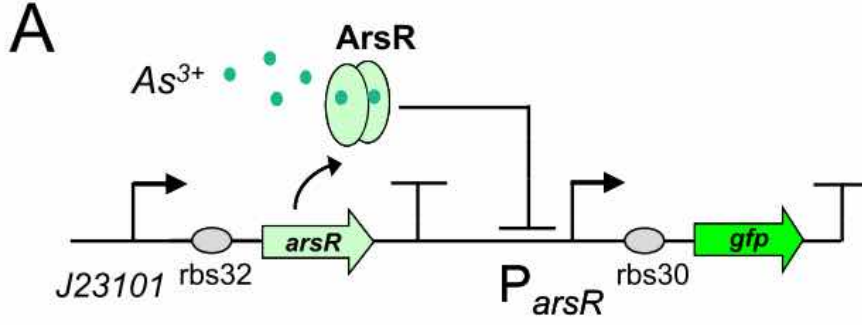


Figure 1: Schematic diagram of plasmid1

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.

$$P_{J23104} \xrightarrow{k_1} P_{J23104} + ArsR \quad (1)$$

$$P_{arsR} \xrightarrow{k_2} P_{arsR} + smURFP \quad (2)$$

$$ArsR + P_{arsR} \xrightleftharpoons[k_{-3}]{k_3} ArsR * P_{arsR} \quad (3)$$

On the plasmid 2, fusion protein of dCas9 (dead Cas9, a mutant of Cas9) and RNAP (RNA polymerase) are produced after transcription.

$$P_{tet} \xrightarrow{k_4} P_{tet} + dCas9 - RNAP \quad (4)$$

$$P_{tet} \xrightarrow{k_5} P_{tet} + sgRNA \quad (5)$$

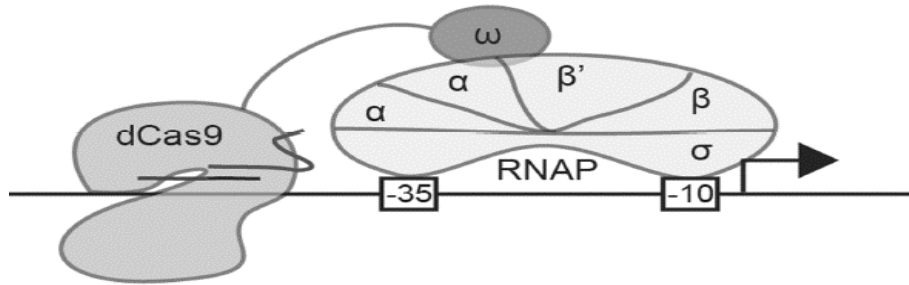


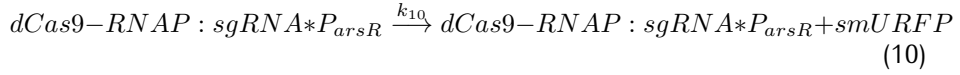
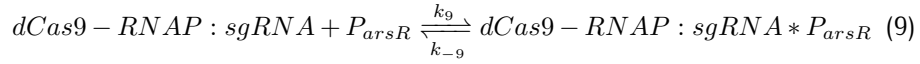
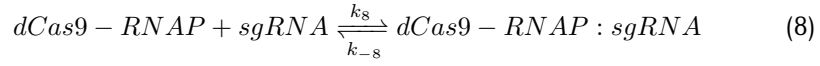
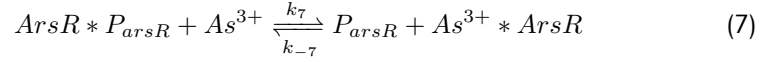
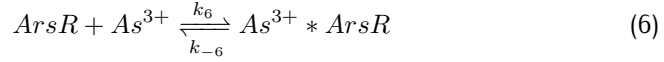
Figure 2: Schematic diagram of dCas9/RNAP

dCas9/RNAP can bind with its 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} .

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



Take the degradation into account

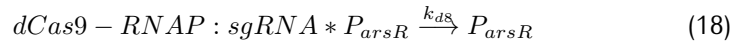
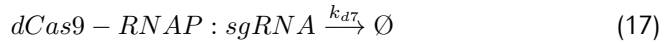
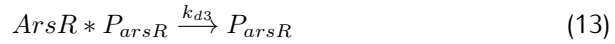


Table 1: Parameters

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

3.2 simulation

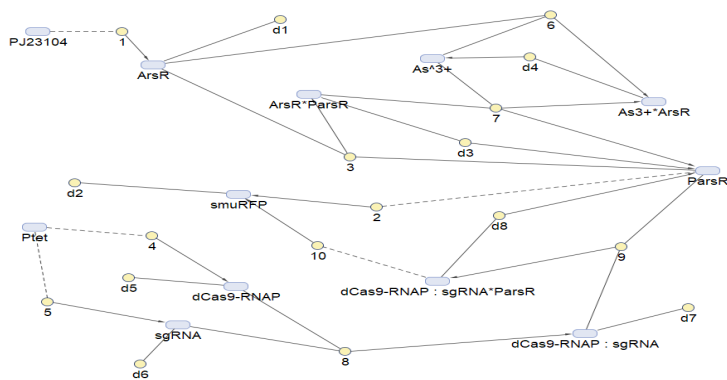


Figure 3: reaction map generated from the reaction set above using SimBiology Toolbox



Figure 4: Schematic diagram of smURFP fluorescence