Labbook of dynamic CORN

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October 3, 2018

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

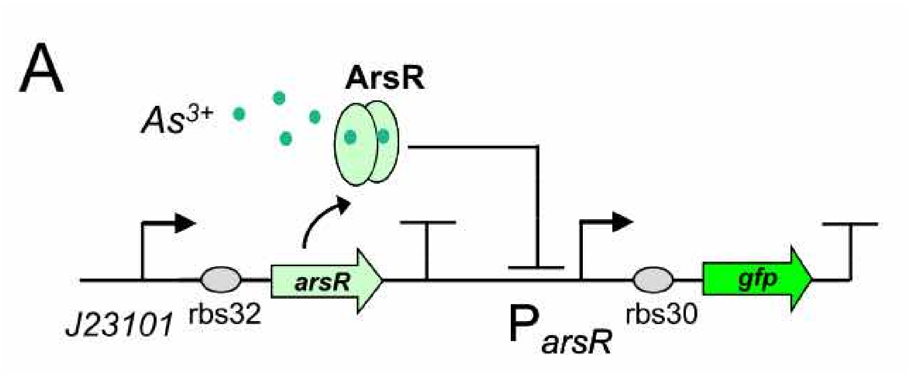

Figure 1: Schematic diagram of plasmid1

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. [**pola2018novel**]

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

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

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

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.

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

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

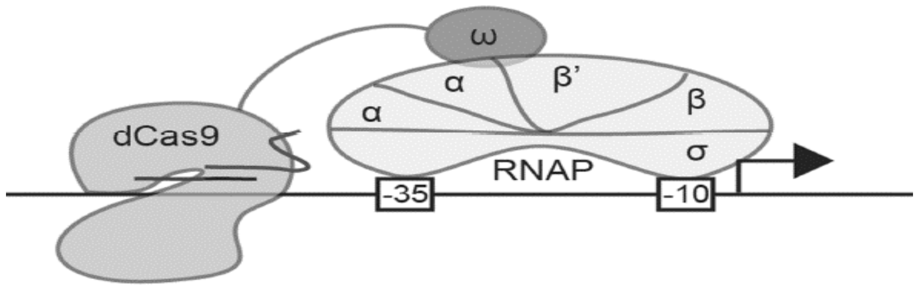

Figure 2: Schematic diagram of dCas9/RNAP

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} .[**bikard2013programmable**]

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:

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

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

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

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

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

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

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

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

We can now consider each reaction(not following the order above). We will use v as an arbitrary variable, simply there to denote a rate of reaction. The r0x notation will be used later in the programming to remove clutter from the code. The several complexes involved:$ArsR * P_{arsR}$, $As^{3+} * ArsR$, dCas9-RNAP, dCas9*RNAP:sgRNA, $dCas9 * RNAP : sgRNA * P_{arsR}$, are respectively abbreviated as $cplx_1$, $cplx_2$, $cplx_3$, $cplx_4$, $cplx_5$.

(1)Production of protein ArsR (r01):

$$v = k_1[P_{J23104}]$$

(2)Degration of ArsR protein (r02):

$$v = -k_{d1}[ArsR]$$

Note that this rate will be negative. This is because it will be decreasing the concentration of the ArsR protein . We can now put them together, to form an ordinary differential equation (ODE) for the rate of change in ArsR protein concentration.

$$\frac{d[ArsR]}{dt} = k_1[P_{arsR}] - k_{d1}[P_{arsR}][ArsR] \tag{1}$$

(3)Production of protein smURFP under unbound promoter (r03):

$$v = k_2[P_{arsR}]$$

(4)Production of protein smURFP under dCas9*RNAP:sgRNA-bound promoter (r04):

$$v = k_{10}[cplx_5]$$

(5)Degration of smURFP protein (r05):

$$v = -k_{d2}[smURFP]$$

These two come together to form an ODE for the rate of change in smuRFP protein concentration:

$$\frac{d[smURFP]}{dt} = k_2[P_{arsR}] + k_{10}[cplx_5] - k_{d2}[smuRFP] \tag{2}$$

(6)Combination of ArsR protein and P_{arsR} promoter(r06):

$$v = k_3[ArsR][P_arsR]$$

(7)Combination of As^{3+} and ArsR protein bound with P_{arsR} promoter , also dissociation of ArsR protein and P_{arsR} promoter(r07):

$$v = k_7[As^{3+}][cplx_1]$$

(8)Degration of ArsR protein in the $ArsR * P_{arsR}$ complex(r08):

$$v = -k_{d3}[cplx_1]$$

Similarly, these three form the ODE for the rate of change in $ArsR * P_{arsR}$ concentration. :

$$\frac{d[cplx_1]}{dt} = k_3[ArsR][P_{arsR}] - k_7[As^{3+}][[cplx_1] - k_{d3}[cplx_1] \tag{3}$$

Note that, in this ODE, k_7 will be negative. This is because it will be decreasing the concentration of $ArsR * P_{arsR}$ complex.
(9)Production of dCas9-RNAP fusion protein (r09):

$$v = k_4[P_{tet}]$$

(10)Degration of dCas9-RNAP fusion protein(r010):

$$v = k_{d5}[cplx_3]$$

(11)Combination of dCas9-RNAP fusion protein and it's sgRNA(r011):

$$v = k_8[cplx_3][sgRNA]$$

These three form the ODE for the rate of change in $dCas9-RNAP$ concentration.

$$\frac{d[cplx_3]}{dt} = k_4[P_{tet}] - k_8[cplx_3][sgRNA] - k_{d5}[cplx_3] \tag{4}$$

Note that, in this ODE, k_8 will be negative. This is because it will be decreasing the concentration of $dCas9 - RNAP$ complex.
(12)Production of sgRNA (r012):

$$v = k_5[P_{tet}]$$

(13)Degration of sgRNA(r013)

$$v = k_{d6}[sgRNA]$$

These two plus r010 come together to form an ODE for the rate of change in sgRNA concentration:

$$\frac{d[sgRNA]}{dt} = k_5[P_{tet}] - k_8[cplx_3][sgRNA] - k_{d6}[sgRNA] \tag{5}$$

Similarly, in this ODE, k_8 will be negative. This is because it will be decreasing the concentration of sgRNA complex.
(14)Combination of As^{3+} and ArsR protein (r014):

$$v = k_6[As^{3+}][ArsR]$$

(15)Degration of ArsR protein in the $As^{3+} * ArsR$ complex(r015):

$$v = -k_{d6}[As^{3+} * ArsR]$$

These two plus r06 come together to form an ODE for the rate of change in $As^{3+} * ArsR$ concentration and an ODE for the rate of change in As^{3+} concentration:

$$\frac{d[cplx_2]}{dt} = k_6[As^{3+}][ArsR] + k_7[As^{3+}][cplx_1] - k_{d4}[cplx_2] \tag{6}$$

Note that, in this ODE, k_7 will be positive,different from ODE(3). This is because it will be increasing the concentration of $As^{3+} * ArsR$ complex.

$$\frac{d[cplx_2]}{dt} = -k_6[As^{3+}][ArsR] - k_7[As^{3+}][cplx_1] \tag{7}$$

(16)Combination of dCas9-RNAP:sgRNA complex and P_{arsR} promoter (r016):

$$v = k_9[cplx_4][P_{arsR}]$$

(17)Degration of dCas9-RNAP:sgRNA complex (r017):

$$v = -k_{d7}[cplx_4]$$

These two plus r010 come together to form an ODE for the rate of change in dCas9-RNAP:sgRNA complex concentration:

$$\frac{d[cplx_4]}{dt} = k_8[cplx_3][sgRNA] - k_9[cplx_4][P_{arsR}] - k_{d7}[cplx_4] \tag{8}$$

Note that, in this ODE, k_8 will be positive,different from ODE(4). This is because it will be decreasing the concentration of dCas9-RNAP:sgRNA complex.
(18))Degration of dCas9-RNAP:sgRNA complex in $dCas9 - RNAP : sgRNA * P_{arsR}$ compex(r018):

$$v = -k_{d8}[cplx_5]$$

This reaction and r016 come together to form an ODE for the rate of change in $dCas9 - RNAP : sgRNA * P_{arsR}$ complex concentration:

$$\frac{d[cplx_5]}{dt} = k_9[cplx_4][P_{arsR}] - k_{d8}[cplx_5] \tag{9}$$

At last, the ODEs for the rate of change in three promoter:

$$\frac{d[P_{J23104}]}{dt} = 0 \tag{10}$$

$$\frac{d[P_{ArsR}]}{dt} = 0 \tag{11}$$

$$\frac{d[P_{tet}]}{dt} = 0 \tag{12}$$

Altogether, we have 8 ODEs:

$$\frac{d[ArsR]}{dt} = k_1[P_{arsR}] - k_{d1}[P_{arsR}][ArsR] \tag{1}$$

$$\frac{d[smURFP]}{dt} = k_2[P_{arsR}] + k_{10}[cplx_5] - k_{d2}[smuRFP] \tag{2}$$

$$\frac{d[cplx_1]}{dt} = k_3[ArsR][P_{arsR}] - k_7[As^{3+}][[cplx_1] - k_{d3}[cplx_1] \tag{3}$$

$$\frac{d[cplx_3]}{dt} = k_4[P_{tet}] - k_8[cplx_3][sgRNA] - k_{d5}[cplx_3] \tag{4}$$

$$\frac{d[sgRNA]}{dt} = k_5[P_{tet}] - k_8[cplx_3][sgRNA] - k_{d6}[sgRNA] \tag{5}$$

$$\frac{d[cplx_2]}{dt} = k_6[As^{3+}][ArsR] + k_7[As^{3+}][cplx_1] - k_{d4}[cplx_2] \tag{6}$$

$$\frac{d[cplx_2]}{dt} = -k_6[As^{3+}][ArsR] - k_7[As^{3+}][cplx_1] \tag{7}$$

$$\frac{d[cplx_4]}{dt} = k_8[cplx_3][sgRNA] - k_9[cplx_4][P_{arsR}] - k_{d7}[cplx_4] \tag{8}$$

$$\frac{d[cplx_5]}{dt} = k_9[cplx_4][P_{arsR}] - k_{d8}[cplx_5] \tag{9}$$

$$\frac{d[P_{J23104}]}{dt} = 0 \tag{10}$$

$$\frac{d[P_{ArsR}]}{dt} = 0 \tag{11}$$

$$\frac{d[P_{tet}]}{dt} = 0 \tag{12}$$

Table 1: Parameters

Rate constants	Value	units	
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

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.

References

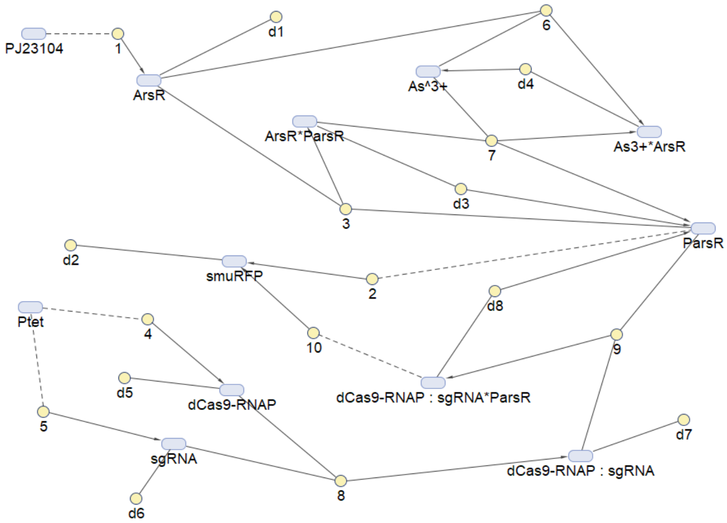

Figure 3: reaction map generated from the reaction set above using SimBiology Toolbox

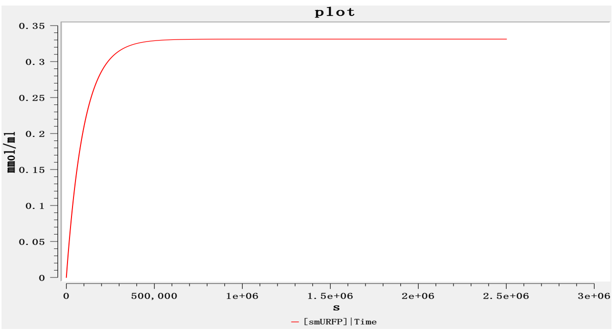

Figure 4: Schematic diagram of smURFP fluorescence by COPASI