Dynamic Model of Heavy Metal Detection Biosensor

Minghui Yin, Sherry Dongqi Bao
Tianjin University

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Abstract

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.

2 Method

2.1 Analysis of reactions

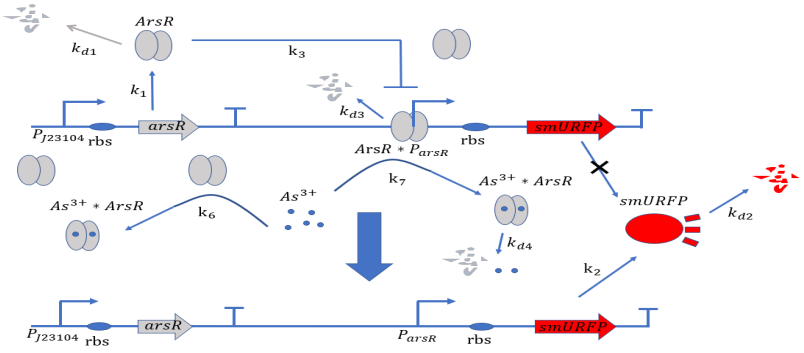

Figure 1: Schematic diagram of plasmid1

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**]

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

$$P_{arsR_d} \xrightarrow{k_2} P_{arsR_d} + \text{smURFP}_l \tag{2}$$

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

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.

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

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

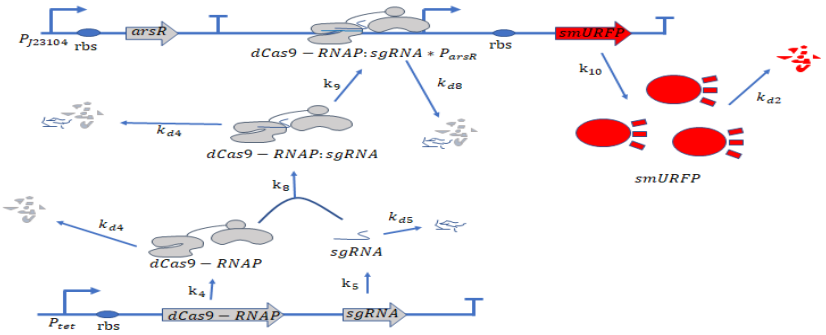

Figure 2: Schematic diagram of dCas9/RNAP

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: $[\text{dCas9/RNAP}] = [\text{dCas9}] = [\text{RNAP}]$; $[P_{arsR_d}] = [P_{arsR_u}] = 0.5[P_{arsR}]$)

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

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

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

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

$$P_{\text{arsR}_\text{d}} \xrightarrow{k_{10}} P_{\text{arsR}_\text{d}} + \text{smURFP} \tag{10}$$

We then take degradation into account:

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

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

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

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

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

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

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

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

2.2 Establishment of ODEs

We can now write each reaction into ODEs (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$_{\text{arsR}}$, As^{3+}*ArsR, dCas9*RNAP, dCas9*RNAP:sgRNA, dCas9*RNAP:sgRNA*P$_{\text{arsR}}$, 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 thatthere should be a minus sign. 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.

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

(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:

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

(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. :

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

Note that, in this ODE, there should be a minus sign in front of k_7. 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.

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

Note that, in this ODE, there should be a minus sign in front of k_8. 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:

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

Similarly, in this ODE,there should be a minus sign in front of k_8 . 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:

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

Note that, in this ODE, there should be no minus sign in front ofk_7 ,which is different from ODE(3). This is because it will be increasing the concentration of $As^{3+} * ArsR$ complex.

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

(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:

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

Similarly, in this ODE, there should be no minus sign in front ofk_8 , which is 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:

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

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

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

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

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

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

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

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

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

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

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

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

Table 1: Parameters

Rate constants	Value	Units	Reference
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

2.3 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.

2.4 Sensitivity Analysis

A good biosystem should be with a stability towards fluctuations in parameters. And a good model should also reflect this and hence a test for robustness can be an important test of the model. Robustness analysis can also pinpoint which

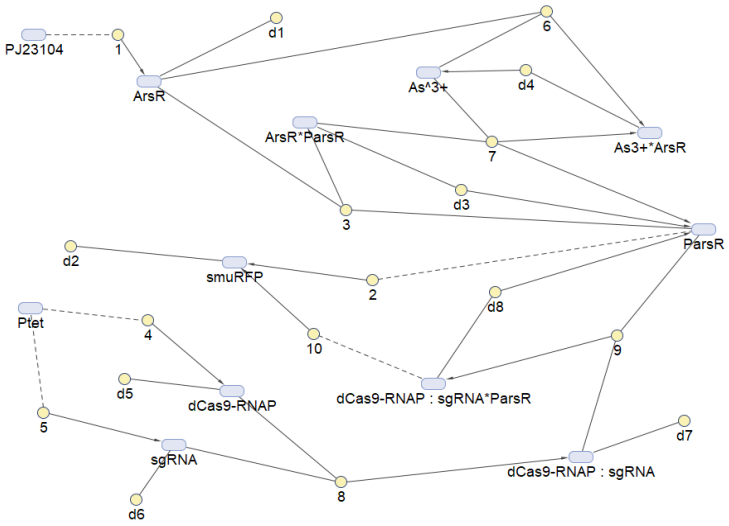

Figure 3: reaction map generated from the reaction set above using SimBiology Toolbox

reactions/parameters that are important for obtaining a specific biological behavior. A simple measure for sensitivity is to measure the relative change of a system feature due to a change in a parameter. As for our model, the feature can be the equilibrium concentration of the smuRFP,C for which the sensitivity (S) to a parameter k is:

$$S = \frac{\frac{dC}{C}}{\frac{dk}{k}} = \frac{dC}{dk}\frac{k}{c} \approx \frac{\Delta C}{\Delta k}\frac{k}{c} \tag{19}$$

The Matlab scripts used for sensitivity analysis see attached.

2.5 Modification of the model

The results are not very satisfactory, and we suspect that it may be due to the simplification of the two processes of transcription and translation when producing proteins into a one-step process.So we take the mRNA into account, and then the reaction (1) to (4) four reactions should be replaced by the following 6 reaction equations:

$$P_{J23104} \xrightarrow{k_{1'}} P_{J23104} + mRNA_{ArsR} \tag{20}$$

$$mRNA_{ArsR} \xrightarrow{k_{2'}} mRNA_{ArsR} + ArsR \tag{21}$$

$$P_{arsR} \xrightarrow{k_{3'}} P_{arsR} + mRNA_{smURFP} \tag{22}$$

$$mRNA_{smURFP} \xrightarrow{k_{4'}} mRNA_{smURFP} + smURFP \tag{23}$$

$$P_{tet} \xrightarrow{k_{5'}} P_{tet} + mRNA_{dCas9-RNAP} \tag{24}$$

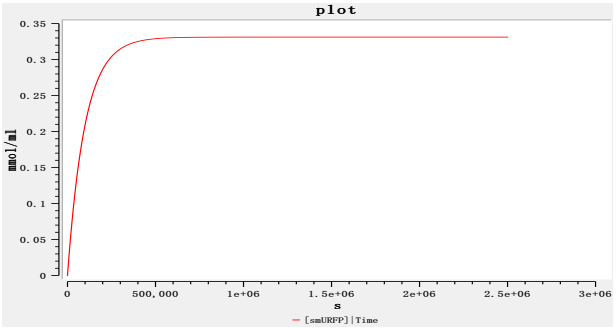

Figure 4: Schematic diagram of smURFP fluorescence by COPASI

$$mRNA_{dCas9-RNAP} \xrightarrow{k_{6'}} mRNA_{dCas9-RNAP} + dCas9 - RNAP \tag{25}$$

And the reaction equation set for the degradation of the reaction substance should also be added to the following two reaction equations.

$$mRNA_{ArsR} \xrightarrow{k_{d9}} \emptyset \tag{26}$$

$$mRNA_{smURFP} \xrightarrow{k_{d10}} \emptyset \tag{27}$$

$$mRNA_{dCas9-RNAP} \xrightarrow{k_{d11}} \emptyset \tag{28}$$

Added parameter values:

2.6 a bold assumption

Since the goal of our project is to try to increase the sensitivity of biosensors by introducing a complex of dcas9-RNAP and sgRNA, and the purpose of our model is to explore whether this complex is effective. So why not assume a reasonable and large enough concentration value for this complex. We use the concentration of glyceraldehyde-3-phosphate dehydrogenase A as the assumed concentration. Glyceraldehyde 3 phosphate dehydrogenase A (gapA) is a key enzyme in the

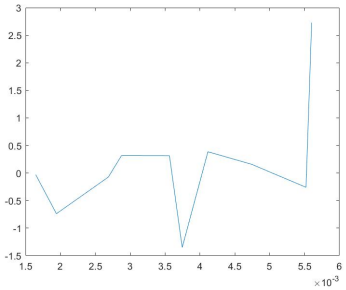

Figure 5: sensitivity of k1

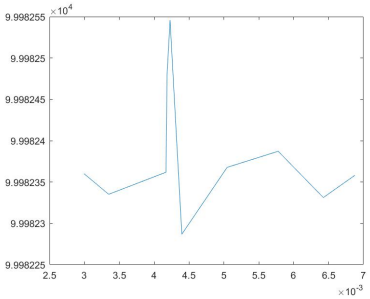

Figure 6: sensitivity of k2

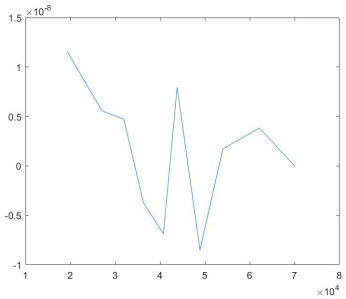

Figure 7: sensitivity of k3

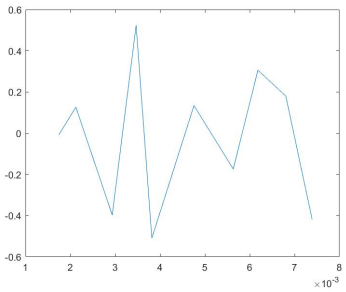

Figure 8: sensitivity of k4

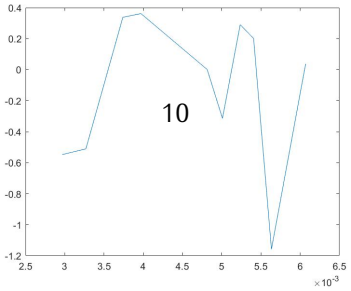

Figure 9: sensitivity of k5

Table 2: Parameters

Rate constants	Value	units	source
k1'	1.5e-2	s^{-1}	Berset et al.
k2'	7.33e-2	s^{-1}	Berset et al.
k3'	1.5e-2	s^{-1}	Berset et al.
k4'	1.84e-13	s^{-1}	Berset et al.
k5'		s^{-1}	
k6'		s^{-1}	
kd9	2.81e-3	ns^{-1}	Berset et al.
kd10	7.62e-3	s^{-1}	Berset et al.
kd11		s^{-1}	

glycolytic pathway, and the gene encoding this enzyme is a housekeeping gene in E. coli cells with high expression levels. We found in the literature that the protein mass of gapA is 48645fg/cell, and its molecular weight is 35492 Da.The amount of abundance of Glyceraldehyde 3 phosphate dehydrogenase A protein per cell can be calculated as follows:

$$n = \frac{m}{M} = \frac{48645 * 10^{-15} g}{35492 g/mol} = 1.37 * 10^{-15} mol$$

As for the size of E. coli, we found relevant data from the literature, see the figure below.

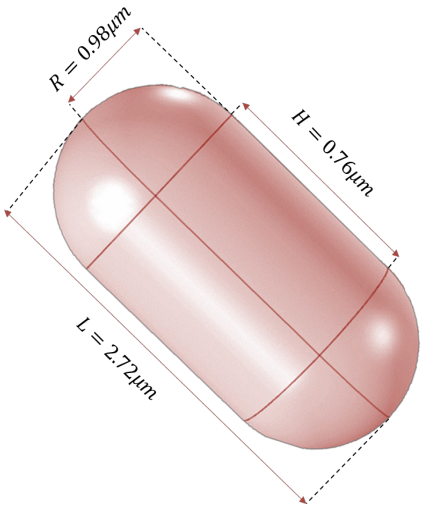

Figure 23: size of E. coli

The volume of E. coli can be calculated as follows:

$$V_{E.coli} = \frac{4}{3}\pi R^3 + \pi R^2 H = \frac{4}{3}\pi(0.98\mu m)^3 + \pi(0.98\mu m)^2(0.76\mu m) = 6.24\mu m^3 = 6.24 * 10^{-15} L$$

Then the concentration of Glyceraldehyde 3 phosphate dehydrogenase A pro-

tein in the cell can be determined:

$$c = \frac{n}{V_{E.coli}} = \frac{1.37 * 10^{-15} mol}{6.24 * 10^{-15} L} = 0.22 mol/L$$

With this concentration, we got very nice results:

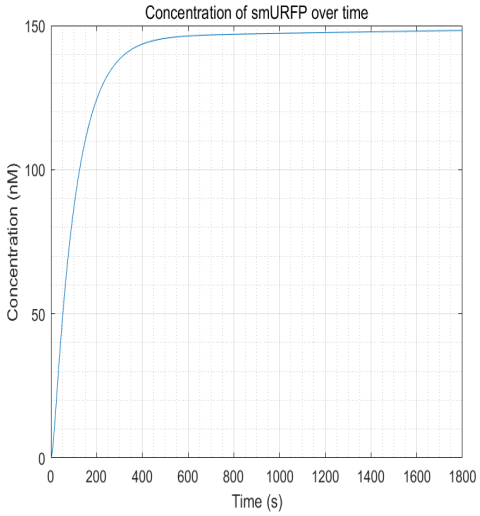

Figure 24: Schematic diagram of smURFP fluorescence

Compared to the diagram without introducing dcas9: From these three fig-

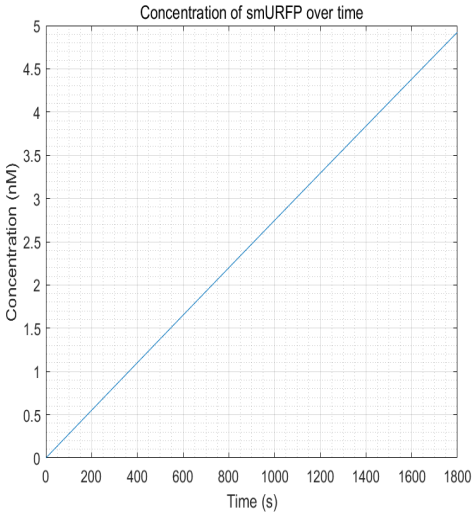

Figure 25: smURFP fluorescence within a reasonable time frame

ures, we can conclude that dCas9-RNAP:sgRNA does have the effect of promoting transcription and increasing fluorescence intensity, thereby increasing sensitivity, as long as its concentration is sufficient. This result enhances the confidence of

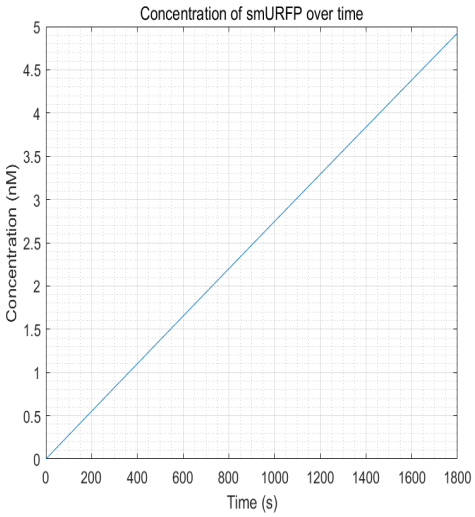

Figure 26: smURFP fluorescence reaches equilibrium but costs too long

the experimental group, they just need to try to improve the expression of dCas9-RNAP:sgRNA in E. coli without having to doubt its role.

References